

AD_____

Award Number: DAMD17-99-2-9025

TITLE: The Associate Program on Ethnobiology, Socio-Economic
Value Assessment and Community Based Conservation

PRINCIPAL INVESTIGATOR: Maurice M. Iwu, Ph.D.

CONTRACTING ORGANIZATION: Bioresources Development and
Conservation Programme
Silver Spring, Maryland 20902

REPORT DATE: October 2001

TYPE OF REPORT: Annual

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

DISTRIBUTION STATEMENT: Approved for Public Release;
Distribution Unlimited

The views, opinions and/or findings contained in this report are those of the author(s) and should not be construed as an official Department of the Army position, policy or decision unless so designated by other documentation.

20021104 093

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 074-0188

Public reporting burden for this collection of information is estimated to average 1 hour per response, including the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing this collection of information. Send comments regarding this burden estimate or any other aspect of this collection of information, including suggestions for reducing this burden to Washington Headquarters Services, Directorate for Information Operations and Reports, 1215 Jefferson Davis Highway, Suite 1204, Arlington, VA 22202-4302, and to the Office of Management and Budget, Paperwork Reduction Project (0704-0188), Washington, DC 20503

1. AGENCY USE ONLY (Leave blank)**2. REPORT DATE**

October 2001

3. REPORT TYPE AND DATES COVERED

Annual (1 Oct 00 - 30 Sep 01)

4. TITLE AND SUBTITLE

The Associate Program on Ethnobiology, Socio-Economic Value Assessment and Community Based Conservation

5. FUNDING NUMBERS

DAMD17-99-2-9025

6. AUTHOR(S)

Maurice M. Iwu, Ph.D.

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)

Bioresources Development and Conservation Programme
Silver Spring, Maryland 20902

E-Mail: iwum@bioresources.org

**8. PERFORMING ORGANIZATION
REPORT NUMBER****9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)**

U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

**10. SPONSORING / MONITORING
AGENCY REPORT NUMBER****11. SUPPLEMENTARY NOTES****12a. DISTRIBUTION / AVAILABILITY STATEMENT**

Approved for Public Release; Distribution Unlimited

12b. DISTRIBUTION CODE

13. ABSTRACT (Maximum 200 Words) In collaboration with the Smithsonian Institution, BDCP conducted training courses in Nigeria and Cameroon on biodiversity assessment and monitoring and an adaptive management workshop in Nigeria. Four I ha biodiversity plots were also established. In the drug discovery component, sixty plant extracts were processed and 12 compounds isolated from plants used in African traditional medicine as antimalaria. The following plants were bulk-extracted at InterCEDD Nsukka, Nigeria: *Aspilia africana*, *Chamaecrista mimosoides*, *Combretum dulchipetalum*, *Enantia chlorantha* and *Pleiocarpa pycnantha* to enable further fractionations at WRAIR. Sixty-nine plant extracts were also processed and tested for potential activity against microorganisms and for activity in the in the brine shrimp lethality assay. Twelve extracts showed very significant activities against gram-positive and/ or gram-negative bacteria and 2 of the extracts showed antifungal activities. Extracts from 11 plants identified through ethnobotanical information were tested for their CNS activity using specific radio ligand binding assays. Extracts were also processed for testing against African trypanosomes, leishmaniasis, HIV, Cystic fibrosis, tuberculosis and cancer. The economic value assessment of selected species is continuing. The analysis of the ethnobotanical data from Cameroon has been completed and the final report will be published in a usable CD format.

14. SUBJECT TERMS

Phytochemistry, Anti-malaria, Plant collection, Fractionation, Database, Biodiversity
Economic evaluation, Medicinal plants, Antiparasitic, Bioassays

15. NUMBER OF PAGES

44

16. PRICE CODE**17. SECURITY CLASSIFICATION
OF REPORT**

Unclassified

**18. SECURITY CLASSIFICATION
OF THIS PAGE**

Unclassified

**19. SECURITY CLASSIFICATION
OF ABSTRACT**

Unclassified

20. LIMITATION OF ABSTRACT

Unlimited

FOREWORD

Opinions, interpretations, conclusions and recommendations are those of the author and are not necessarily endorsed by the U.S. Army.

X Where copyrighted material is quoted, permission has been obtained to use such material.

X Where material from documents designated for limited distribution is quoted, permission has been obtained to use the material.

X Citations of commercial organizations and trade names in this report do not constitute an official Department of Army endorsement or approval of the products or services of these organizations.

N/A In conducting research using animals, the investigator(s) adhered to the "Guide for the Care and Use of Laboratory Animals," prepared by the Committee on Care and use of Laboratory Animals of the Institute of Laboratory Resources, national Research Council (NIH Publication No. 86-23, Revised 1985).

N/A For the protection of human subjects, the investigator(s) adhered to policies of applicable Federal Law 45 CFR 46.

N/A In conducting research utilizing recombinant DNA technology, the investigator(s) adhered to current guidelines promulgated by the National Institutes of Health.

N/A In the conduct of research utilizing recombinant DNA, the investigator(s) adhered to the NIH Guidelines for Research Involving Recombinant DNA Molecules.

N/A In the conduct of research involving hazardous organisms, the investigator(s) adhered to the CDC-NIH Guide for Biosafety in Microbiological and Biomedical Laboratories.

W. M. M. W. 10-1-01
PI - Signature Date

Table of Contents

Cover..1
SF 298..2
Introduction..5
Body..6
Key Research Accomplishments..6
References..44

INTRODUCTION:

This project was executed under the collaborative agreement between the Fogarty International Center of the National Institutes of Health and Walter Reed Army Institute of Research as part of the International Collaborative Biodiversity Groups (ICBG). The ICBG project, jointly sponsored by the U.S. National Institutes of Health, the National Science Foundation and the U.S. Department of Agriculture has the main focal point of establishing an integrated program for the discovery of biologically active plants for drug development and biodiversity conservation, while ensuring that source countries derive maximum benefits for their biological resources and their intellectual contribution. The African ICBG, in general emphasizes three major goals: evaluation of rainforest plants from Nigeria and Cameroon as cures for parasitic diseases; research on forest dynamics to understand the effects of sustainable harvesting and cultivation of important medicinal plants; training of Cameroonians and Nigerians in natural products chemistry and tropical ecology.

The Bioresources Development and Conservation Programme (BDCP) facilitates the African based drug discovery and economic development components of the ICBG and therefore serves as a link between the drug discovery part of the program, the biodiversity conservation component and the economic development projects.

The aims of the Associate Programs administered by BDCP are:

1. To conduct ethnobiological inventory of plants in the selected study areas;
2. To guide the ICBG in its plant selection and collection strategies for drug discovery. Samples identified from ethnobiological inventory will be collected from biodiversity plots and from wild flora and screened for possible biological activity.
3. To perform phytochemistry and preliminary bioassays on selected plants.
4. To perform plant extraction, bioassay-guided isolation, structural elucidation with research training and infrastructure development being important components of each operation.
5. To maintain and expand the database on African medicinal plants, which includes information on local names, traditional uses, floristic data, possible constituents, conservation status, agronomic data and economic value. This involved the re-structuring and expansion of the existing AfricMed database to include data from other Associate Programs. This Computerized Information System of African Medicinal Plants (CISAMAP) will be linked to other regional databases.
6. To conduct a socio-economic value assessment of the biological resources in the study area which seeks to:
 - I) highlight the non-commercial value of forest products within the cultural/ religious context;
 - II) quantify the economic value of biological resources for comparison with other land use options;
 - III) place in priority order the production and marketing of biological resources in local markets to provide income for local residents;
 - IV) provide baseline agronomic data for the formulation of a sustainable management plan for the forest resources; and
 - V) train local natural resource managers and users at the National and Community levels to conduct economic and market research which will integrate the connection between conservation and development. The ICBG may organize rural farmers to cultivate, in fallow areas, certain plants of potential therapeutic value;
7. Assist in capacity building of West African scientists in the areas of ethnobiology, inventory and research management. Formal training will be organized in ethnobiological methods and field taxonomy and economic value assessment for local communities.

BODY:

1. PHYTOCHEMISTRY AND PRELIMINARY BIOASSAYS

1.1 Specific Aims:

- i. Conduct extraction and bioassay guided separation of African plants used in ethnomedicine for treatment of target diseases
- ii. Isolate and characterize the bioactive constituents of these plants
- iii. Perform bulk extraction and fractionation of plant material for *in vivo* assays
- iv. Optimize lead compounds with significant potential for human health in Africa
- v. Provide training for African scientists in phytochemistry and drug development

1.2 Progress Report/ Key Research Accomplishment:

1.2.1 Phytochemistry

Extract Preparation: Several plants used locally for the treatment of malaria were harvested and extracted using standard methods. A total of 60 extracts (Tables 1 and 2) were obtained from these studies. Thirty-six of these were obtained from Cameroon while the rest were prepared at International Centre for Ethnomedicine and Drug Development (InterCEDD) in Nigeria.

TABLE 1

ICBG PLANT SAMPLES SUBMITTED FOR *IN VITRO* ANTIMALARIAL (RM) SCREENING

Lab No	Plant name	Extractcode
SU-2052	<i>Homalium letestui</i>	HL1
SU-2053	<i>Schefflera</i> spp	Abo
SU-2054	<i>Khaya anthotheca</i>	TKA
SU-2055	<i>Aframomum sulcatum</i>	ADH
SU-2056	<i>Euphorbia poinsonii</i>	EPA
SU-2057	<i>Euphorbia kinnii</i>	EKA
SU-2058	<i>Euphorbia eutorrofilla</i>	EEA
SU-2059	<i>Anogeissus leiocarpus</i>	ALE1
SU-2060	<i>Anogeissus leiocarpus</i>	ALE2
SU-2061	<i>Anogeissus leiocarpus</i>	ALE3
SU-2062	<i>Lannea acida</i>	LPA
SU-2063	<i>Inula klingii</i>	IK
SU-2064	<i>Terminalia superba</i>	TST
SU-2065	<i>Terminalia glaucescens</i>	TGC1
SU-2066	<i>Terminalia glaucescens</i>	TGC2
SU-2067	<i>Terminalia superba</i>	TST2
SU-2068	<i>Aframomum sceptrum</i>	ASS
SU-2069	<i>Garcinia kola</i>	GSP
SU-2070	<i>Ellophobia</i> spp	ELS1
SU-2071	<i>Ellophobia</i> spp	ELS2
SU-2072	<i>Anisopus mannii</i>	ANIM
SU-2073	<i>Combretum glutinosum</i>	CCG

SU-2074	<i>Combretum aculeatum</i>	CCA
SU-2075	<i>Pteleopsis hylodendron</i>	MPH1
SU-2076	<i>Pteleopsis hylodendron</i>	MPH2
SU-2077	<i>Pteleopsis hylodendron</i>	MPH3
SU-2078	<i>Pteleopsis hylodendron</i>	MPH4
SU-2079	<i>Vitellaria paradoxa</i>	PVA
SU-2080	<i>Renealmia porypus</i>	REA
SU-2081	<i>Renealmia porypus</i>	PRAA
SU-2082	<i>Albizia ferruginea</i>	AAF
SU-2083	<i>Marantochloa purpurea</i>	MPA
SU-2084	<i>Marantochloa purpurea</i>	MPAC
SU-2085	<i>Penianthus longifolius</i>	PLA
SU-2086	<i>Penianthus longifolius</i>	PLE
SU-2087	<i>Crotalaria incana</i>	CIA
SU-2113	<i>Eugenia uniflora</i> fraction	EUH
SU-2114	<i>Eugenia uniflora</i> fraction	EUI
SU-2115	<i>Eugenia uniflora</i> fraction	EUJ
SU-2116	<i>Eugenia uniflora</i> fraction	EUK
SU-2117	<i>Gongronema latifolium</i> Fr.	GLA
SU-2118	<i>Gongronema latifolium</i> Fr.	GLB
SU-2119	<i>Gongronema latifolium</i> Fr.	GLC
SU-2120	<i>Gongronema latifolium</i> Fr.	GLD
SU-2121	<i>Gongronema latifolium</i> Fr.	GLE

Table 2

Plant Name	Part of Plant Used	Extraction Solvent
<i>Solanum torvum</i>	Fruit	Methylene Chloride
<i>Mussaenda elegan</i>	Stem	Methylene Chloride
<i>Vernonia migeodii</i>	Aerial parts	Methylene Chloride
<i>Hyptis suaveolens</i>	Leaf & stem	Methylene chloride
<i>Dorstenia multiradiata</i>	Whole Plant	Methylene chloride
<i>Paropsia guineensis</i>	Leaf	Methylene Chloride
<i>Mussaenda elegan</i>	Stem	Methanol
<i>Vernonia migeodi</i>	Aerial parts	Methanol
<i>Hyptis suaveolens</i>	Leaf & Stem	Methanol
<i>Portulaca oleracea</i>	Whole plant	Methanol
<i>Dorstenia multiradiata</i>	Whole plant	Methanol
<i>Paropsia gueneensis</i>	Leaf	Methanol
<i>Voacanga africana</i>	Leaf	Water
<i>Gossypium arboreum</i>	Aerial parts	Water
<i>Pycnanthus angolensis</i>	Stem bark	Water
<i>Sida acuta</i>	Leaf	Water
<i>Olex subscorpoidea</i>	Root	Water
<i>Mussaenda elegan</i>	Stem	Water
<i>Lophira lanceolata</i>	Root	Water
<i>Gnetum</i>	Leaf	Water
<i>Adenopus senegalensis</i>	Seed coat	Water
<i>Erythrina senegalensis</i>	Root	Water
<i>Eupatorium odoratum</i>	Leaf	Water

1.3. PRELIMINARY BIOASSAYS

1.3.1 Brine Shrimp Lethality

Of the total of 22 extracts which were studied for their cytotoxicity against brine shrimp nauplii, 5, 4, 6, 3 and 4, representing 22.7, 18.2, 27.3, 13.6 and 18.3 per cents of the total were active at < 50, 51 – 100, 101 – 500, 501–1000, and > 1000 µg/ml respectively. Of the first category, the most active extract was that from *Combretum racemosum* WP (H₂O) which, at an LD₅₀ of 1.21µg/ml as significantly the most active extract (Table 3).

Table 3. BRINE SHRIMP CYTOTOXICITY ASSAY RESULTS

Plant extracts	LD ₅₀ (µg/ml)
1. <i>Caesaria barterii</i> L (CH ₃ OH)	297.47
2. <i>Cola nitida</i> L (H ₂ O)	58.39
3. <i>Pycnanthus angolensis</i> S (H ₂ O)	328.43
4. <i>Guarea cedrata</i> S/B (CH ₂ Cl ₂)	7248.56
5. <i>Ritchea longipediculata</i> L (CH ₂ Cl ₂)	> 5.0x 10 ⁹
6. <i>Pycnanthus angolensis</i> S (CH ₃ OH)	144.44
7. <i>Mussandra elegans</i> L (CH ₃ OH)	> 5.0x 10 ⁹
8. <i>Chlorophora excelsa</i> L (CH ₃ OH)	725.69
9. <i>Trichillia emetica</i> L (CH ₂ Cl ₂)	282.73
10. <i>Chlorophora excelsa</i> L (CH ₂ Cl ₂)	540.86
11. <i>Grewia cissoides</i> S (CH ₃ OH)	40.23
12. <i>Treculia africana</i> L (CH ₂ Cl ₂)	557.75
13. <i>Dissotis rotundifolia</i> L/S (H ₂ O)	62.46
14. <i>Picralima nitida</i> S (H ₂ O)	183.2
15. <i>Napoleona imperialis</i> L (H ₂ O)	1252.34
16. <i>Erythrina senegalensis</i> R (H ₂ O)	66.79
17. <i>Cassia siamea</i> L (H ₂ O)	149.72
18. <i>Morinda lucida</i> L (H ₂ O)	81.69
19. <i>Combretum racemosum</i> W/P (H ₂ O)	1.21
20. <i>Garcinia cola</i> L (H ₂ O)	39.15
21. <i>Dennetia tripetalia</i> L (H ₂ O)	40.23
22. <i>Picralima nitida</i> L (H ₂ O)	39.15

1.3.2 ANTIMICROBIAL BIOASSAYS.

A total of 69 extracts of various plants were screened for activity against microbes. As shown (Table 4), only twelve (12) extracts displayed any antimicrobial activity. Nine extracts consisting of *Trichillia emetica* L (CH₂Cl₂), *Chlorophora excelsa* L (CH₃OH), *Treculia africana* L (CH₂Cl₂), *Guarea cedrata* S (CH₂Cl₂), *Dennetia tripetala* L (H₂O), *Acanthus montanus* L (H₂O), *Hyptis suaveolens* L (CH₂Cl₂), *Pycnanthus angolensis* SB (H₂O) and *Protea madiensis* L (H₂O) were active against multiple bacteria. The *Treculia africana* L (CH₂Cl₂) extract was particularly noteworthy in that it displayed broad-spectrum antimicrobial activity against both Gram positive and Gram-negative bacteria, as well as yeast.

Table 4. Results of antimicrobial screening.

Plant extract	S aureus	B. subtilis	E coli	Ps. aeruginosa	C albicans
<i>Grewia cissoides</i> stem (H ₂ O)	-	-	-	-	-
<i>Napoleona imperialis</i> L (H ₂ O)	+	+	-	-	-
<i>Guarea cedrata</i> S/B (CH ₂ Cl ₂)	+	-	+	-	-
<i>Casia siamea</i> L (H ₂ O)	-	-	-	-	-
<i>Dennetia tripetala</i> L (H ₂ O)	+	-	+	-	-
<i>Erythrina senegalensis</i> R (H ₂ O)	-	-	-	-	-
<i>Picralima nitida</i> seeds (H ₂ O)	-	-	-	-	-
<i>Pleiocarpa pycnantha</i> L (H ₂ O)	-	-	-	-	-
<i>Pleiocarpa pycnantha</i> L (H ₂ O)	-	-	-	-	-
<i>Carica papaya</i> L (H ₂ O)	-	-	-	-	-
<i>Newbouldia laevis</i> L (H ₂ O)	-	-	-	-	-
<i>Cola nitida</i> L (CH ₂ Cl ₂)	-	-	-	-	-
<i>Persia Americana</i> seed (CH ₂ Cl ₂)	-	-	-	-	-
<i>Synclesia scabride</i> L (H ₂ O)	-	-	-	-	-
<i>Bucholzia coriacea</i> seed (CH ₂ Cl ₂)	-	-	-	-	-
<i>Picralima nitida</i> R (H ₂ O)	-	-	-	-	-
<i>Gouania longipetala</i> L/S (H ₂ O)	-	-	-	-	-
<i>Trichilia emetica</i> L (CH ₂ Cl ₂)	+	-	+	-	+
<i>Cola nitida</i> L (H ₂ O)	-	-	-	-	-
<i>Caesaria barterii</i> L (CH ₃ OH)	-	-	-	-	-
<i>Chlorophora excelsa</i> L (CH ₂ Cl ₂)	-	-	+	+	+
<i>Dissotis rotundifolia</i> L/S (H ₂ O)	-	-	-	-	-
<i>Garcinia kola</i> L (H ₂ O)	-	-	-	-	-
<i>Chlorophora excelsa</i> L (CH ₃ OH)	-	-	-	-	+
<i>Garcinia kola</i> R (H ₂ O)	-	-	-	-	-
<i>Combretum racemosum</i> W/P (H ₂ O)	-	-	-	-	-
<i>Treculia africana</i> L (CH ₂ Cl ₂)	+	+	-	+	+
<i>Ficus capensis</i> L (CH ₂ Cl ₂)	-	-	-	-	-
<i>Costus lucaniusianus</i> L (H ₂ O)	-	-	-	-	-
<i>Rauwolfia vomitoria</i> R (H ₂ O)	-	-	-	-	-
<i>Myranthus arborieus</i> L (CH ₂ Cl ₂)	-	-	-	-	-
<i>Ritchea longipedicilata</i> L (CH ₂ Cl ₂)	-	-	-	-	-
<i>Treculia africana</i> L (CH ₂ Cl ₂)	+	+	-	-	-
<i>Guarea cedrata</i> S (CH ₂ Cl ₂)	+	+	-	-	+
<i>Amaranthus spinosus</i> AP (CH ₃ OH)	-	-	-	-	-
<i>Moringa oleifera</i> L (CH ₂ Cl ₂)	-	-	-	-	-
<i>Cassia siamea</i> L (H ₂ O)	-	-	-	-	-
<i>Treculia africana</i> L (CH ₂ Cl ₂)	-	-	-	-	-
<i>Ritchea longipedicula</i> L (CH ₂ Cl ₂)	-	-	-	-	-
<i>Ritchea longipedicula</i> L (CH ₃ OH)	-	-	-	-	-
<i>Voacanga africana</i> L (H ₂ O)	-	-	-	-	-
<i>Solenostemon monostachys</i> WP (H ₂ O)	-	-	-	-	-
<i>Gossypium arboreum</i> AP (H ₂ O)	-	-	-	-	-
<i>Olex subscorpoidea</i> R (H ₂ O)	-	-	-	-	-
<i>Dissotis rotundifolia</i> L/S (CH ₃ OH)	-	-	-	-	-

<i>Chlorophora excelsa</i> L (H ₂ O)	-	+	-	-	-
<i>Trichillia emetica</i> L (CH ₂ Cl ₂)	+	-	-	-	-
<i>Costus lucamusianus</i> L (H ₂ O)	-	-	-	-	-
<i>Dennetia tripetala</i> L (H ₂ O)	+	+	-	-	-
<i>Acanthus montanus</i> L (H ₂ O)	+	-	+	-	+
<i>Protea madiensis</i> L (H ₂ O)	+	+	+	-	+
<i>Mussandra elegans</i> L(CH ₂ Cl ₂)	-	-	-	-	-
<i>Pycnanthus angolensis</i> S/B (H ₂ O)	-	-	-	-	+
<i>Voacanga africana</i> L (H ₂ O)	-	-	-	-	-
<i>Dissotis rotundifolia</i> L (CH ₃ OH)	-	-	-	-	-
<i>Olex subscorpoidea</i> R (H ₂ O)	-	-	-	-	-
<i>Gossipium arboreum</i> A/P (H ₂ O)	-	-	-	-	-
<i>Ritchea longipediculata</i> L (CH ₂ Cl ₂)	-	-	-	-	-
<i>Vernonea migeodii</i> A/P (CH ₂ Cl ₂)	-	-	-	-	-
<i>Cola nitida</i> L (H ₂ O)	-	-	-	-	-
<i>Caesariia barterii</i> L (CH ₃ OH)	-	-	-	-	-
<i>Byrsocarpus coccinens</i> L (H ₂ O)	-	-	-	-	-
<i>Chlorophora excelsa</i> L (CH ₃ OH)	-	-	-	-	-
<i>Hyptis suaveolens</i> L (CH ₂ Cl ₂)	+	+	-	-	+
<i>Morinda lucinda</i> L (H ₂ O)	-	-	-	-	-
<i>Dissotis rotundifolia</i> L (H ₂ O)	-	-	-	-	-
<i>Pycnanthus angolensis</i> seeds (H ₂ O)	-	-	-	-	-
<i>Picralima nitida</i> L (H ₂ O)	-	-	-	-	-
<i>Combretum racemosum</i> W/P (H ₂ O)	-	-	-	-	-

For screening, the extracts were employed at a concentration of 2000µg/ml. Inability of the test organism to grow at this concentration after 24 to 48 h was adjudged to mean that the extract had anti-microbial activity.

Extracts showing activity in preliminary bioassays were tested further to determine their potencies. The latter (presented as minimum inhibitory concentrations, MICs) are shown in **Table 5** for 20 extracts. Extracts from *Combretum dulcipetalum* were particularly active, displaying significant activity against all the bacteria as well as against the yeast *Candida albicans*. Two of the extracts which displayed significant antifungal properties (*Dracaena mannii* (CH₃OH) and *Combretum dulcipetalum* (CH₃OH)) are undergoing fractionation with aim of isolating and characterizing the bioactive constituents.

Table 5. Minimum inhibitory concentrations (MICs) of active extracts.

Plant extract	MIC ($\mu\text{g/ml}$) against				
	<i>S aureus</i>	<i>B. subtilis</i>	<i>E coli</i>	<i>Ps. aeruginosa</i>	<i>C albicans</i>
1. <i>Sida cordifolia</i> L (H_2O)	2000	2000	2000	2000	-
2. <i>Combretum dulcipetalum</i> L (H_2O)	1000	-	-	-	-
3. <i>Celosia trigyna</i> W/P (H_2O)	-	-	-	-	2000
4. <i>Combretum dulcipetalum</i> L (CH_2Cl_2)	-	-	-	-	2000
5. <i>Ocimum gratissimum</i> L/S (CH_2Cl_2)	-	500	-	-	-
6. <i>Piptadeniastrum africanum</i> S/B (CH_3OH)	500	-	-	-	-
7. <i>Dennetia tripetala</i> L (CH_2Cl_2)	-	-	-	-	2000
8. <i>Combretum dulcipetalum</i> R (CH_3OH)	-	-	-	-	500
9. <i>Dracaena mannii</i> S (CH_2Cl_2)	-	-	-	-	2000
10. <i>Dracaena mannii</i> S (CH_3OH)	-	-	-	-	1000
11. <i>Napoleona imperialis</i> L (H_2O)	1000	1000	-	-	-
12. <i>Combretum dulcipetalum</i> R (H_2O)	1000	1000	1000	1000	-
13. <i>Chlorophora excelsa</i> L (CH_2Cl_2)	500	250	-	-	-
14. <i>Trichillia emetica</i> L (CH_2Cl_2)	-	31.125	-	-	-
15. <i>Erythrina senegalensis</i> L (CH_2Cl_2)	500	62.5	500	2000	-
16. <i>Cassia siamea</i> L (H_2O)	-	500	1000	-	-
17. <i>Ficus capensis</i> L (CH_2Cl_2)	32.5	250	2000	-	-
18. <i>Combretum racemosum</i> L/S (CH_2Cl_2)	-	-	250	-	-
19. <i>Myranthus arboreum</i> L (CH_2Cl_2)	-	-	-	-	2000
20. <i>Costus lucamusianus</i> L (H_2O)	-	-	2000	-	-

- Means not tested against.

1.3.4 Lead Optimization/Organic Synthesis.

Previous studies have identified cryptolepine as the bioactive constituent of the antimalarial plant *Cryptolepine sanguinolenta*. However, this compound is known to intercalate DNA and may not be optimal for long-term use. In effort to evaluate the activity of related compounds, two cryptolepine analogues were synthesized in 500mg lots and submitted for testing.

Bioassay-directed Fractionation of extracts of *Araliopsis tabouensis*

Araliopsis tabouensis Aubrev & Pellegr (Rutaceae) is used in the traditional medicine of West and Central African countries against fevers and malaria. It is a large evergreen tree found throughout the region that is also used in the treatment of sexually transmitted diseases. Our earlier investigation indicated that *A. tabouensis* possessed strong antiplasmodial activity (Okunji *et al* 2000) as well as being active against four strains of *Trypanosoma* and *Trichomonas*. A detailed phytochemical study of this plant yielded 22 compounds. The structures of these compounds were determined by standard methods. Extracts of the stem bark of *A. tabouensis* and twenty-three alkaloids isolated from the extracts were examined *in vitro* against *Plasmodium falciparum*. Our previous

investigation was based on classical phytochemistry without the incorporation of bioassay. However subsequent bioassay of the methanol extract demonstrated the highest activity against both strains of *Plasmodium*, while the hexane and aqueous extracts were less active. The 50% inhibitory concentration (IC_{50}) values for the methanol extract ranged between 0.895 microgram/ml ($\mu\text{g/ml}$) and 1.042 $\mu\text{g/ml}$. Among the isolated compounds, Araliopdimerine-A (AT3), a minor quinolone alkaloid of *A. tabouensis*, was shown to exhibit the highest growth-inhibitory activity against the malarial parasite with IC_{50} values of 0.034 $\mu\text{g/ml}$ and 0.176 $\mu\text{g/ml}$ against D6 and W2, respectively. Other alkaloids, including vepresine and N-methylpreskimmianine, were moderately active. Three heptacyclic dimeric 2-quinoline alkaloids (verpridimerineA, B & C) and araliopsinnine were inactive.

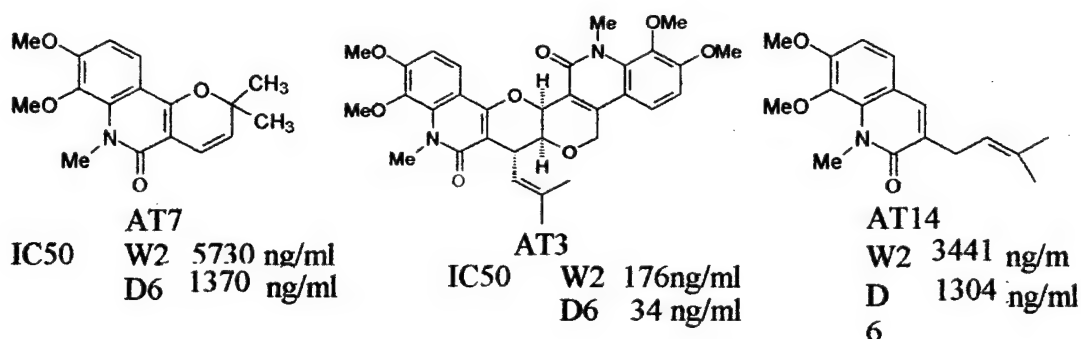


Fig. 4

AT3 is 100 and 70 times more potent than AT7 and AT14, respectively, against the D-6 clone. In addition, AT3 is 54- and 35-fold more potent than AT7 and AT14, respectively, against the chloroquine-resistant W-2 clone.

Similarly, the methanol extract of the stem bark of this plant exhibited antitrypanosomal activity against four strains of *Trypanosoma brucei in vitro*. Vepresine was found to possess pronounced activity against the four strains of *T. brucei* with IC_{50} values between 25.5 and 100 $\mu\text{g/ml}$. Against this background, the purpose of present study is to carry out a systematic bioassay-directed chromatographic separation of active extracts from *A. tabouensis* in order to isolate the antiprotozoal agents.

Using a combination of Sephadex-LH20 gel filtration, column chromatography on silica gel, HPLC and preparative TLC provided an efficient dereplication tool for the isolation of AT3 at AT7 and AT14. Although AT3 is the most potent antimalarial constituent isolated from *A. tabouensis*, this compound is also one of the most structurally complex of these constituents, which might make total synthesis of AT3 very difficult and expensive. However, unlike other natural product compounds, the yields of AT series are very encouraging.

Starting from 40g of extract of *A. tabouensis* stem bark, we have able to develop a reproducible protocol which yielded 100mg/40g of stem bark of AT3 and 1000mg/40g of AT7. Out of the 110 plant samples from AP2 submitted for antimalarial testing, only the results of 30 samples were available, while others are pending.

In continuation of our investigation on *Pachypodanthium staudtii*, we had previously isolated six compounds whose antimalarial activity was less potent than fractions

processed through bioassay-guided fractionation. Therefore, bioassay directed fractionation of the most active methylene chloride extract from *P. staudtii* stem bark was carried out. As shown in Table 6, Flash column separation of methylene chloride extract from *P. staudtii* stem bark yielded seven bulked fractions whose antimalarial activity is shown in Table 6. The most active fraction (PS1; fr 1-13) was eluted with chloroform and gave an IC_{50} values of 130.97ng/ml for D6 and 88.7 for W2 respectively. Other fractions with remarkable activity include PS2, PS3 and PS7 giving IC_{50} values ≥ 220 nanogram/ml. The results revealed that chromatographic fractions from active extracts demonstrated significant higher antimalarial activity than those observed with the crude extracts. As shown in Table 6 some of the seven fractions of *P. staudtii* were equipotent against chloroquine-susceptible and chloroquine-resistant strains of *P. falciparum*. Furthermore, all fractions were highly active particularly against the chloroquine-resistant strain (W₂) of *P. falciparum*. Therefore promising antimalarial lead structures could be developed from this plant species commonly used in African traditional medicine for the treatment of malaria.

Plant based commercial samples used clinically in Nigeria for the treatment of resistant malaria were purchased at Abuja, Nigeria during the International Conference on Traditional Medicine in HIV/AIDS and Malaria for testing against malarial parasites. The samples include Nigerian Bitter, which is liquid preparation in waterproof sachets and Ajadilopea Antimalarial spice (powder). The two samples were processed to yield five extracts by successive extraction/partition with organic solvent and the aqueous portion lyophilized. The samples were submitted for *in vitro* test against two strains of *P. falciparum*. The results are shown in Table 6. The maximum inhibitions were observed in organic extract of Ajadilopea Antimalarial spice. Promising antiprotozoal activity with IC_{50} values of 4.8 and 80.5 ng/ml against D₆ and W₂ strains of *P. falciparum* respectively. It is concluded that such product with very low IC_{50} values may serve as sources of lead compounds with therapeutic potency. The present results showed that medicinal plants, which are used in traditional medicine against malaria, might have some antimalarial activity.

Scoparia dulcis as Plant-Derived Proton Pump Inhibitors

Scoparia dulcis was investigated for presence of compounds capable of targeting the proton pumps of *P. falciparum*. *Scoparia dulcis* (Fam. *Scrophulariaceae*) is a perennial herb, which is distributed in tropical and subtropical regions. It is popularly used in traditional medicine for the treatment malaria and fever.

Our earlier *in vitro* assay of extracts from *Scoparia dulcis* showed good antimalarial activity with IC_{50} values of 1562 and 1202 ng/ml against D₆ and W₂ strains of *P. falciparum* respectively. In collaboration with Dr. Reil, of Medical Pharmacology Fellowship program at WRAIR. Scopadulcic acids A and B are known to be active components of this plant.

A directed search based on selecting plant derivatives known to have proton pump inhibitory activity has not been systematically applied. The biological rationale for this study was based on the ubiquitous nature of proton pumps in eukaryotic cells. P-type and V-type proton pumps are present on the plasma membrane of malaria parasites. These pumps are required for the maintenance of optimal cytosolic pH. The food vacuole has a

V-type proton pump that is bafilomycin A1 sensitive. The acidocalcisome has a V-type proton pyrophosphatase that is a plant-like pump. The V-type proton pyrophosphatase is not found in humans.

The chemical rationale for this study was based on the fact that scopadulcic acid A, scopadulcic acid B and scopadulciol are the main constituent of this plant. According to Hayashi (1991), a methyl ester of scopadulcic acid B showed the most potent inhibitory activity of gastric proton pumps. In 1999, Overman reported enantiodivergent total synthesis of (+) and (-) scopadulcic acid A, one of the tetracyclic diterpenes found in *Scoparia dulcis* having weaker H⁺, K⁺-ATPase inhibitory activity. *Plasmodium falciparum* has H⁺-ATPase in the plasma membrane and food vacuole and an H⁺-PPase in the acidocalcisome. These proton pumps are potential targets for antimalarial therapy and may have their function disrupted by compounds known to inhibit gastric proton pumps.

We sought to determine the IC₅₀ of Scopadulcic acid A *in vitro* as a test of concept. The methylene chloride extract of *S. dulcis* was fractionated by gel filtration using Sephadex LH-20. The column was eluted with chloroform: methanol mixtures. A total of four bulk fractions were collected and evaporated to dryness. About 5mg each of these fractions together with methylene chloride extract serving as standard were submitted for *in vitro* antimalarial test against D6, W2 and C235 the mefloquine multi-resistant strains of *P. falciparum*.

The results showed *in vitro* activity with an IC₅₀ of 27 micromolar for the D6 strain and an IC₅₀ of 18 micromolar for the W2 strain of *P. falciparum*. The log dose response curve obtained against the C235 strain was not interpretable. We submit that additional testing of this class of compounds is warranted and may lead to a new class of drugs for the treatment of malaria.

Table 6: IC₅₀ values of fractions of plant extracts tested against two clones of *Plasmodium falciparum*, one sensitive to chloroquine (D6) and another chloroquine-resistant (W2).

SAMPLE	Synonym	Lab No	Target	ng/ml	<=>	*IC ₅₀
	Chloroquine	D6	1000	=		14.69
	Chloroquine	W2	1000	=		388.442

Pachypodanthium staudtii CH₂Cl₂ Flash Column fractions

PS1 (Fr. 1-13)	BP22282	SU-1975	D6	5000	=	130.978
	BP22282		W2	5000	=	88.783
PS2 (Fr. 14-21(79-92))	BP22291	SU-1976	D6	5000	=	182.262
	BP22291		W2	5000	=	142.323
PS3 (Fr. 22-36)	BP22308	SU-1977	D6	5000	=	545.255
	BP22308		W2	5000	=	453.001
PS4 (Fr. 37-56)	BP22317	SU-1978	D6	5000	=	1897.878
	BP22317		W2	5000	=	930.028
PS5 (Fr. 57-107)	BP22326	SU-1979	D6	5000	>	5000

	BP22326		W2	5000	=2092.948
PS6 (Fr. 108-)	BP22335	SU-1980	D6	5000	>5000
	BP22335		W2	5000	>5000
PS7	BP22344	SU-1981	D6	5000	=219.9
	BP22344		W2	5000	=114.752
<i>Araliopsis tabouensis</i>	BP22353	SU-2026	D6	5000	>5000
LH-20 Fr. 1-3	BP22353		W2	5000	>5000
Fr. 4	BP22362	SU-2027	D6	5000	>5000
	BP22362		W2	5000	=1446.091
Fr. 8	BP22371	SU-2028	D6	5000	=1534.413
	BP22371		W2	5000	=988.442
Fr. 10-23	BP22380	SU-2029	D6	5000	=1573.473
	BP22380		W2	5000	=934.144
Fr. 16-26	BP22399	SU-2030	D6	5000	=1643.021
	BP22399		W2	5000	=1062.123
CC silica gel Fr. 24-60	BP22306	SU-2031	D6	5000	=2928.624
	BP22306		W2	5000	=1847.388
Fr. 1-11	BP22415	SU-2032	D6	5000	=1482.045
	BP22415		W2	5000	=738.912
Fr. 12-31	BP22424	SU-2033	D6	5000	=1602.001
	BP22424		W2	5000	=847.628
Fr. 96-110	BP22433	SU-2034	D6	5000	=1412.441
	BP22433		W2	5000	=689.339
Fr. 136-160	BP22442	SU-2035	D6	5000	>5000
	BP22442		W2	5000	=1963.253
Fr. 136-160	BP22451	SU-2036	D6	5000	=1108.417
	BP22451		W2		768.17
Fr. 179-196	BP22460	SU-2037	D6	5000	=2205.537
	BP22460		W2	5000	=1238.572
Fr. 197	BP22479	SU-2038	D6	5000	=2089.865
	BP22479		W2	5000	=1604.579
Fr. 198	BP22488	SU-2039	D6	5000	=2786.959
	BP22488		W2	5000	=1824.945
Fr. 200	BP22497	SU-2040	D6	5000	=2196.444
	BP22497		W2	5000	=955.202
Fr. 111-135	BP22504	SU-2041	D6	5000	=1359.653

	BP22504		W2	5000	=228.963
Ajadilopea: Antimalarial spice clinical sample					
Aqueous fraction	BP22513	SU-2042	D6	5000	>5000
	BP22513		W2		550.17
organic extract A	BP22522	SU-2043	D6	5000	>4.883
	BP22522		W2	5000	= 80.527
organic extract B	BP22531	SU-2044	D6	5000	>9.766
	BP22531		W2	5000	>156.25
Nigerian Bitter clinical antimalarial sample					
Ethyl acetate extract	BP22540	SU-2045	D6	5000	=884.017
	BP22540		W2	5000	>1250
Lyophilized ext (untreated)	BP22559	SU-2046	D6	5000	=1413.677
	BP22559		W2	5000	>19.531
<i>Picralima nitida</i>					
SU-371 fractionationfr.11	BP22568	SU-2047	D6	5000	>5000
	BP22568		W2	5000	>5000
HSCCC fr. 10	BP22577	SU-2048	D6	5000	=342.877
	BP22577		W2	5000	>5000
HSCCC fr. 61	BP22586	SU-2049	D6	5000	= 666.883
	BP22586		W2	5000	= 629.959
HSCCC fr. 64	BP22595	SU-2050	D6	5000	=724.843
	BP22595		W2	5000	>5000
HSCCC fr. 3	BP22602	SU-2051	D6	5000	=141.474
	BP22602		W2	5000	>5000
<i>Lophira lanceolata</i>					
Fr.18-31	BP20617	SU-1972	D6	16072.6	
			W2	14971.6	
<i>Lophira lanceolata</i> Fr.49-59	BP20626	SU-1973	D6	>25000	
			W2	15132.9	
<i>Lophira lanceolata</i> (MeOH st bark)	BP20635	SU-1974	D6	24002.8	
			W2	23414.8	
<i>Lophira lanceolata</i> (MeOH rt bark)	BP20644	SU-1975	D6	>50000	
			W2	>50000	
Control	MEFLOQUINE		D6	250	=7.771
	MEFLOQUINE		W2	250	=2.649

*IC₅₀ values in nanogram/ml.

1.4 Antitrypanosomes and Antitrichomonads:

a) African trypanosomes. *In vitro* screens with bloodstream form trypanosomes are set up in 24 well plates using duplicate wells of 4 extract concentrations (in HMI medium) each + full-growth controls, as detailed in Bacchi et al (1996). Initial wide concentration curves were followed by narrow-ranging curves to determine IC₅₀ values. Strains of trypanosomes used were: *Trypanosoma brucei*, Lab 110 EATRO (veterinary parasite); *Trypanosoma rhodesiense* KETRI 243 (human

isolate), *T. rhodesiense* 243 As 10-3 (clone of KETRI 243 highly resistant to melarsoprol and pentamidine).

b) Trichomonads. The method used was the minimal inhibitory concentration (MIC) assay developed by Meingassner et al (1978). Strains used were *T. vaginalis* C1-NIH (ATTC 30001) and a metronidazole-resistant strain, CDC-085 (ATCC 50143). These are aerobically in 96 well plates with triplicate serial dilutions, and checked at 24 and 48 h.

Results

a) Trypanosomes. A total of 39 plant extracts from InterCEDD and an additional 6 received from the University of Dschang were screened against three strains of African trypanosomes. Another eight extracts from InterCEDD were screened against *Trichomonas vaginalis* isolates and 18 were tested against the veterinary parasite *Tritrichomonas foetus*.

Of the 39 extracts received from InterCEDD, all were tested vs. *T. brucei* Lab 110 EATRO and *T. rhodesiense* KETRI 243, and 38 were tested vs. the drug-resistant clone, KETRI 243.

The following extracts had sufficient activity ($IC_{50} \leq 20 \mu\text{g/ml}$) against one or more isolates to warrant further consideration for *in vivo* testing or further purification: SU#: 1863, 1866, 1869, 1870-1876, 1878-1881, 1886, 1889, and 1891. These samples were obtained from the following plants: *Aspilia africana*, *Chamaecrista mimosoides*, *Combretum dulchipetalum*, *Cryptolepis sanguinolenta*, *Enantia chlorontha*, *Hoslundia opposita*, *Icacina trichanta*, *Phyllanthus amarus*, *Pleiocarpa pycnantha*, *Trimfetta tomentosa*, and *Uvaria chamae*. Those extracts having activity at $< 5 \mu\text{g/ml}$ were: SU#: 1872, 1874, 1878, 1880, 1891. We regarded these as prime candidates for *in vivo* trial and have done preliminary *in vivo* tests with these extracts.

Twenty-six extracts were screened against the three isolates and reported in the previous report. We received an additional nine extracts for *in vitro* screening and have partial results with six of these. Two of the six, ASP and TZM, show high activity ($0.5-2.35 \mu\text{g/ml}$).

b) Trichomonads. A total of eight InterCEDD extracts were tested against *T. vaginalis* extracts *in vitro* (Table 9). Of these SU: 1863, 1870, 1873, and 1877 had MIC values $< 1 \text{ mg/ml}$, and are considered of interest for further study. This screen was limited because of continuing growth and contamination problems with the *Trichomonas* isolates. Extracts 1866-1902 (a total of 22) were screened for activity vs. *Tritrichomonas foetus*, a veterinary parasite for which there is no cure. Of these 1874 and 1895 had MIC values of $0.3-0.6 \text{ mg/ml}$, and are considered of interest for further study.

c) In vivo testing. A total of 18 plant extracts gave IC_{50} values of $\leq 20 \mu\text{g/ml}$ in the trypanosome screen. Since many of the extracts were oils, which had to be solubilized in DMSO, we had on hand highly concentrated stock solutions of the extracts. Using 50% DMSO to dilute these stocks, we set up *in vivo* experiments in the *T. brucei* Lab EATRO mouse mode. Dose curves were 1, 5, 10, 25 mg/kg/day i.p. once daily for 3 days. Three mice 25-30 g were used per dose point, and three were untreated, infected controls. The extracts tested were: SU1863, 1866-1876, 1879-1881, 1886, 1889-1891. None were curative, although SU1875 and 1891, prolonged the lifespan of 10 and 25 mg/kg groups by 2-3 days beyond that of the infected, untreated controls. Doses higher than 25 mg/kg were not possible because the volume of 50% DMSO given would have needed to be increased to a toxic level. SU1889 was highly toxic at doses $> 1 \text{ mg/kg}$.

1.5 Antiviral:

This report outlines the antiviral evaluations of twenty-six (26) plant extracts for Walter Reed Army Institute of Research by Southern Research Institute.

SU2009: This compound has an IC_{50} of 6.2 $\mu\text{g/ml}$. However its antiviral activity is limited by compound cytotoxicity with a TC_{50} of 38.8 $\mu\text{g/ml}$.

SU2012 has an IC_{50} at 38.10. It just barely fails to reach a TC_{50} , but examination of the curves shows that above 62.5 $\mu\text{g/ml}$ the compound is becoming cytotoxic and antiviral activity is paralleling cytotoxicity.

SU2016: In contrast to *SU2012* there is no significant cytotoxicity at 200 $\mu\text{g/ml}$ for this compound. Therefore cytotoxicity and antiviral activity appear unrelated.

SU2018: This compound has the same problem as *SU2012*. It starts to show cytotoxicity at 62.5 $\mu\text{g/ml}$ and above this concentration cytotoxicity and antiviral activity parallel each other.

SU2022: This compound is cytotoxic above 20 $\mu\text{g/ml}$ (TC_{50} 41.3 $\mu\text{g/ml}$) with antiviral activity limited by cytotoxicity. However the IC_{50} is about the same as the other compounds, which is consistent.

SU2023: This compound barely reaches an IC_{50} and is highly cytotoxic (TC_{50} 19.2 $\mu\text{g/ml}$).

SU2024: This compound also barely reaches an IC_{50} . However the compound is significantly less cytotoxic than *SU2023* and approaches a TC_{50} at 200 $\mu\text{g/ml}$.

SU2025: This compound barely reaches 50% protection (IC_{50} 169.0 $\mu\text{g/ml}$) in the presence of cytotoxicity (TC_{50} >200 $\mu\text{g/ml}$). This suggests an active component in the mixture, but it is in the minority. In comparison to *SU2024* it is about 3 fold less potent.

Table 7: Summary of HIV Cytoprotection Assay Results

Compound	CEMSS/RF EC_{50} ($\mu\text{g/ml}$)	CEMSS TC_{50} ($\mu\text{g/ml}$)	Therapeutic Index
AZT (μM)	0.003	>1.0	>312.5
SU2000	>200.0	26.5	---
SU2001	>200.0	123.0	---
SU2002	>200.0	122.0	---
SU2003	>200.0	22.4	---
SU2004	>200.0	38.8	---
SU2005	>200.0	13.1	---
SU2006	>200.0	39.2	---
SU2007	>200.0	13.3	---
SU2008	>200.0	13.5	---
SU2009	6.3	33.2	5.3
SU2010	>200.0	13.9	---
SU2011	>200.0	12.5	---
SU2012	38.1	>200.0	>5.3

SU2013	>200.0	1.6	---
SU2014	>200.0	16.4	---
SU2015	>200.0	20.0	---
SU2016	27.0	>200.0	>7.4
SU2017	>200.0	16.4	---
SU2018	35.8	197.0	5.5
SU2019	>200.0	17.7	---
SU2020	>200.0	43.2	---
SU2021	>200.0	50.5	---
SU2022	8.6	41.3	4.8
SU2023	5.9	19.2	3.3
SU2024	55.9	>200.0	>3.6
SU2025	168.0	>200.0	>1.2

1.6 Cystic Fibrosis:

Cystic fibrosis (CF) is an autosomal recessive genetic disease that affects one in each 2000 people in North America (1). The gene affected codes for Cystic Fibrosis Transmembrane Conductance Regulator (CFTR) a member of the ABC transporter family of structurally related proteins that transport diverse compounds across the cell membranes (2-6). The main characteristic feature of this family is the highly conserved nucleotide-binding domain (NBD). Progress has been achieved in prolonging the CF patient's life expectancy from early childhood to over 30 years, but there is still no cure for the lung and pancreas destruction (1).

The CFTR gene encodes a cAMP-stimulated chloride channel (7-15). The mutation associated with most cases of CF, μ F508, is located at the amino-terminal NBD of CFTR (NBD1) (16-17). The CFTR μ F508 mutant is targeted to degradation by the protein quality control, before completing its route through the secretory pathway to the plasma membrane where it would function as a Cl-channel (18-26). A drug that would overcome this protein processing defect could develop into an effective treatment for cystic fibrosis.

In order to search for plant compounds that would have such an activity in the cells of CF patients, bioassays were developed where the molecular defect of CFTR μ F508 was simplified and reproduced in yeast, as explained below. Plants that test positive for the desired activity in yeast are submitted to bioassay-directed fractionation and secondary screening for correction of the CFTR μ F508 processing/function defect in mammalian cells.

Research Accomplishments

Yeast strains produced in Dr. Teem's laboratory at Florida State University were used in a bioassay to detect potential cystic fibrosis therapeutics in tropical plants in Central Africa. The yeast strains are designed to model the molecular defect responsible for cystic fibrosis, and are thus suitable for drug screening assays to identify compounds that reverse the defect. Personnel trained in the use of the assay from Dr. Teem's laboratory traveled to our laboratories in Nigeria and Cameroon with the materials needed to perform the bioassay. Plants from the tropical forest were screened using the assay, and those that are found to contain active substances were identified and retested. Personnel within the host countries were trained in the use of the bioassay. Candidate plants that are identified by the bioassay were used for the preparation of extracts at BDCP laboratory at the University of

Dschang in Cameroon. Active fractions were isolated in Dschang laboratory, and sent to Dr. Teem's laboratory at Florida State University, where they were tested in secondary assays to measure the activity of small molecules on function of the cystic fibrosis transmembrane conductance regulator chloride channel.

Results

The primary screening goals of this project were largely completed in the previous year (May 1999-May 2000), in which plants in Nigeria and Cameroon were screened for activity to correct the cystic fibrosis molecular defect using a yeast two-hybrid bioassay (29). But the results were confirmed during this project period. Numerous candidate plants were identified, and bioassay directed fractionation was conducted. BDCP completed purification of one active compound from a *Trichilia* species at the University of Dschang, Cameroon. The active compound, TS3, was subsequently tested in a secondary mammalian cell assay to determine whether it demonstrated activity to correct defective chloride efflux from mammalian cells expressing the mutant CFTR chloride channel.

Characterization of TS3 in secondary assays

The secondary assay used to test the effectiveness of TS3 is a sensitive electrophysiological assay that quantifies cAMP-stimulated chloride efflux from cells expressing CFTR Δ F508, the mutant chloride channel present in cystic fibrosis patients. Using this assay, the low level of cAMP-stimulated chloride currently associated with the mutant CF mammalian cell line (Fisher Rat Thyroid, FRT) was determined and used as a baseline (30,31). Cells incubated with 5 μ M TS3 demonstrated an 80% increase in chloride efflux as compared to the baseline (no TS3), indicating that the TS3 compound had activity to reverse the molecular defect associated with the mutant CFTR Δ F508 chloride channel. The TS3 compound thus corrected the CF defect in yeast and also in mammalian cells, validating the screening strategy.

The 80% increase in chloride efflux mediated by TS3 is significant, but still small in comparison to chloride efflux associated with normal (non-CF cells). However, full restoration of function is probably not required in order to ameliorate disease in CF patients. Further, the observed level of enhancement mediated by TS3 is approximately equivalent to the level of enhancement observed for other experimental CF therapeutics, such as CPX and genistein. CPX is currently in phase II clinical trials, suggesting that observed activity of TS3 to correct the CF defect is considered clinically relevant.

The enhanced chloride efflux in mammalian cells mediated by TS3 was attained at a concentration of 5 μ M. Concentrations of TS3 greater than 5 μ M were toxic to the cells and precluded a dose-response curve to test the effect of higher concentrations of the compound. The high toxicity of TS3 limits its usefulness as a CF therapeutic lead. However, the positive results obtained with TS3 suggest that other compounds that have activity to correct the CF defect will be identified based upon the yeast bioassay. A subset of these new candidates may have lower toxicity.

Characterization of active fractions of *Hyptis* in secondary assays

As a result of our initial primary screening of Nigerian plant extracts an extract from *Hyptis suaveolens* was identified with activity to reverse the CF defect in the yeast bioassay. This extract had previously been fractionated for another purpose; so limited amounts of fractionated material were available for testing in the yeast bioassay and also in the secondary assay involving mammalian cells. Because only small quantities of *Hyptis* fractions were available, we were not able to perform sufficient numbers of secondary assays to adequately assess the potential of *Hyptis*.

However, preliminary electrophysiological assays on CF cells incubated with *Hyptis* fractions demonstrated a 50% increase in cAMP-stimulated chloride conductance over baseline. These initial positive results, in light of our positive results with TS3, suggests that further bioassay directed fractionation from *Hyptis* is warranted.

1.7 CNS Activity

The primary objective of these studies was to determine the activity of certain plant fractions at monoamine transporters. The approach was to use specific radioligand binding assays to assess the selectivity profiles at dopamine and serotonin transporters.

Background

In our initial investigation, we have selected plants indicated in ethnomedicine for mental illness. Dr. Mash's laboratory at the School of Medicine, University of Miami focused on the dopamine and serotonin transporters, two molecular targets that have been implicated in a variety of neuropsychiatric disorders. Radioligand binding assays were employed in a two-tier protocol. In the first tier, extracts obtained from selected plants were screened to identify potential candidates for further characterization. Extracts were screened at two concentrations: 0.8 µg/tube and 80 µg/tube. Extracts showing 50% or greater inhibitory activity at the highest concentration tested were selected for further characterization. All others were not evaluated in full competition assays. From our preliminary screen for activities, we found that 75% of the plants submitted for testing showed at least 50% inhibitory activity at monoamine transporters.

Materials:

Table 7A summarizes the samples that were determined to be active in preliminary two point assay screens. These compounds demonstrated an inhibitory potency greater than 50% (80 µg/ml stock concentration) at [³H]WIN35,428 binding (DA transporter) and [¹²⁵I]RTI-55 binding (serotonin transporter).

Table 7A: ICBG PLANT SAMPLES WITH RELEVANT POTENCY IN TWO POINT ASSAY SCREENS

In House Sample#	SU-Lab Number	Plant Part	Wt given (mg)
ICBG 1	SU1904	Whole Plnt	25
ICBG 2	SU1905	Sd Pulp	25
ICBG 4	SU1907	Lf/Stem	25
ICBG 8	SU1911	Stbk	25
ICBG 9	SU1912	Whole Plnt	25
ICBG 10	SU1913	Whole Plnt	25
ICBG 11	SU1914	Ft pulp	25

Discussion:

The observation that these plant extracts interact with monoamine transporters prompted an examination of the literature on their biological activities in a search for insights into bioactive constituents. A brief profile of each plant is presented below.

Cassytha filiformis L. (Lauraceae): This plant has been previously found to display alpha adrenoceptor-mediated vasorelaxant activity. In addition, extracts of this plant display inhibitory effects on platelet aggregation induced by a variety of agents. However, the effect of this plant on monoaminergic systems has not been reported. Phytochemical studies of *C. filiformis* have identified a number of bioactive constituents including aporphines, oxoaporphines, a morphinanedienone, lignans and phytosterols. [Some of these same compounds have also been found in *Annona purpurea* (Annonaceae).] Apomorphine, a member of the aporphine family, binds to dopamine receptors and is known as a dopamine agonist. The structure of the aporphine contains the aminoethylphenyl fragment, a characteristic of many compounds that are active at monoaminergic terminals. The aporphines and oxaporphines can therefore be regarded as logical candidates to pursue in subsequent investigations. However, no reports of aporphines as inhibitors of monoamine reuptake have been published. Therefore, it is entirely possible that this aspect of biological activity may be attributed to another constituent.

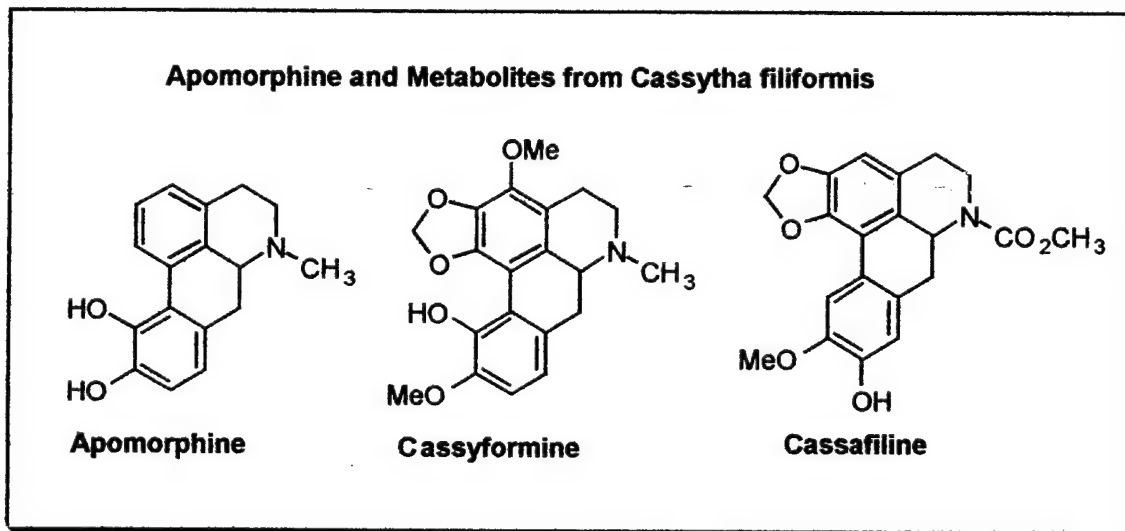


Fig. 5

C. filiformis is used in ethnomedicine as a diuretic, and to treat kidney ailments, gonorrhea, and mental illness. The observation that extracts of this plant can inhibit monoamine reuptake also suggests that *C. filiformis* may also be used to treat depression. Additional investigation of this plant is therefore warranted.

Picralima nitida (Apocynaceae): In African traditional medicine, this plant is used for a variety of indications including malaria, fevers, pain and mental illness. Extracts of this plant have been found to exhibit antiplasmodial, antitrypanocidal and antileishmanial activity. The crude extract of seeds of this plant has been found to exhibit significant antinociceptive activity, which is only partially reversed by naloxone. Phytochemical studies of this plant resulted in the isolation of more than 10 indole alkaloids, including alstonine (also found in *Alstonia boonei*), akuammine, akuammicine, akuammigine, pseudoakuammigine and pericine. Some of these compounds are opioid agonists

while others are opioid antagonists (Menzies et al., 1998); Arens et al., 1982; however, none displays subtype selectivity. Akuammigine is also a weak alpha-adrenoceptor antagonist (Demichel & Roquebert, 1984).

Studies in rats suggest that alstonine is an antipsychotic agent. Similar to typical antipsychotics, alstonine inhibits amphetamine-induced lethality and apomorphine-induced stereotypy, and potentiates barbiturate-induced sleeping time. However, unlike typical antipsychotic agents, the compound also prevents haloperidol-induced catalepsy and fails to bind to dopamine D1, D2 and 5-HT_{2a} receptors. A curious finding in this study, the bell-shaped dose-response curve associated with the effects of alstonine on amphetamine-induced lethality, may be indicative of multiple underlying neurochemical mechanisms. Given the interesting results obtained with the metabolites of *P. nitida*, it is reasonable to suggest that further investigation of its constituents may yield potentially useful insights into the management of mental illness.

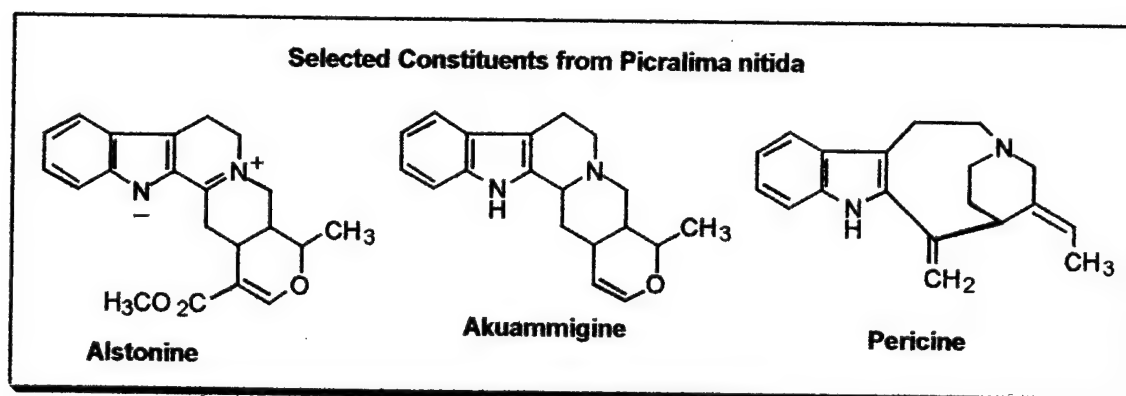


Fig. 6

Triumfetta tomentosa: While this plant is also used in African traditional medicinal for the treatment of mental illness, we could find no reference to other uses in the literature. However, the closely related *Triumfetta rhomboidea*, has been reported to display properties similar to oxytocin (Osone, 1982). In view of its activity on monoamine reuptake system, it would appear that use of this *T. tomentosa* in the treatment of mental illness is not entirely unfounded. Since the extract appears to be highly selective for the dopamine transporter, a combined phytochemical-neurochemical investigation has the potential of identifying a novel selective DAT inhibitor.

Ancistrocladus barteri (Ancistrocladaceae): Plants belonging to the genus *Ancistrocladus* have attracted a great deal of attention since the demonstration of antiviral activity in *A. korupensis* extracts. Subsequent phytochemical studies of this plant have identified the anti-HIV activity to a class of dimeric naphthylisoquinoline alkaloids, notably michellamine B (Boyd et al., 1994). Both the dimeric naphthylisoquinolines and the corresponding monomers (from *Ancistrocladaceae* and *Dioncophyllaceae*) also exhibit varying levels of antimalarial activity (Francois et al., 1997; Hallock et al., 1997 & 1998). However, no reports of testing for targets in the central nervous system have been found. The present observation of moderate inhibition of dopamine reuptake thus serves to expand the pharmacological profile of naphthylisoquinolines, assuming our observations are attributable to the alkaloids present in the extract. Certainly, the naphthylisoquinolines appear to be strong candidates for additional testing in view of the structural similarity between these alkaloids and the well known dopamine reuptake inhibitor nomifensine. Given the apparent selectivity for the dopamine transporter, additional studies may provide further insights into the molecular determinants of selectivity among the monoamines.

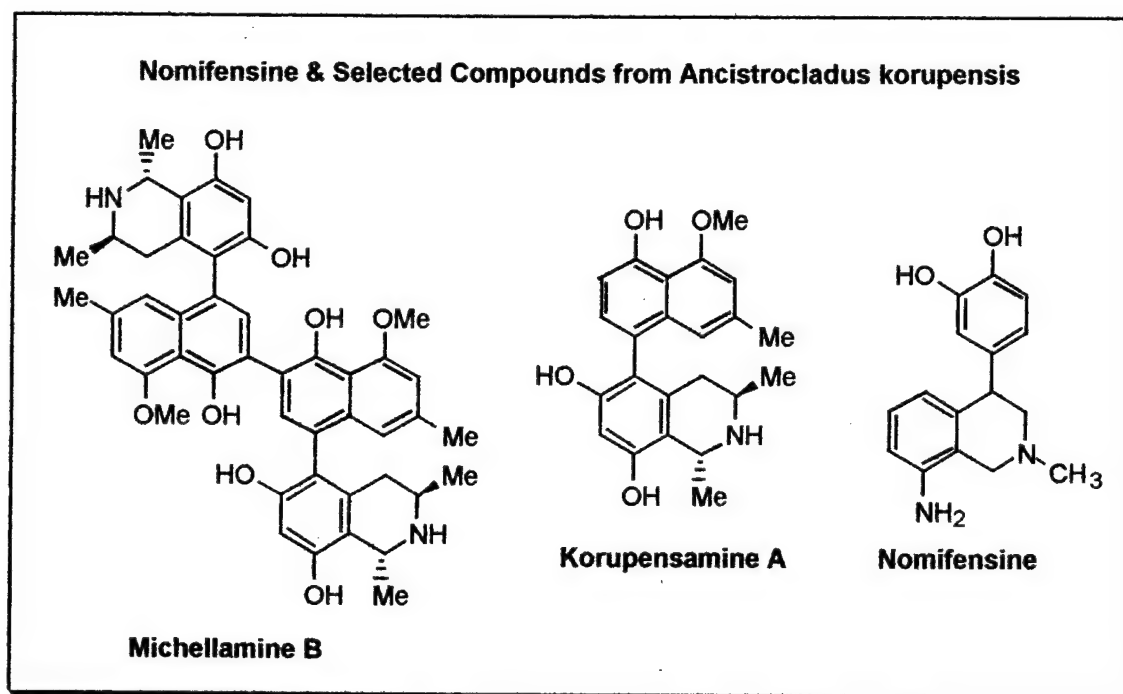


Fig. 7

Scoparia dulcis L. (Scrophulariaceae): In addition to being used to treat mental illness in Africa, this plant is also used in South America (where it commonly known as vassourinha) for a number of indications including pain, fever, bronchitis and gastric disorders. In Oriental medicine *S. dulcis* is used to treat hypertension (Chow et al., 1974). An ethanol extract of this plant has been shown to inhibit binding to dopamine and serotonin receptors (Hasrat et al., 1997). Phytochemical studies have isolated several constituents, among them adrenaline and noradrenaline. These catecholamines probably account for the sympathomimetic effects observed in animals following administration of an extract of the plant (Freire et al., 1996). However, our observations are not easily explained. As shown in Table MMM, the methanolic extract of *S. dulcis* selectively inhibited radioligand binding to DAT; however, the methylene chloride extract was equally effective at both DAT and SERT. Since methylene chloride would not extract the catecholamines, the observation of activity in this fraction points to the presence of another active constituent. Additional investigation is therefore warranted.

Dracaena mannii: Plants belonging to the genus *Dracaena* are used in ethnomedicine for a variety of ailments. A review of the literature finds that steroidal saponins, flavonoids and chalcones have been isolated from this plant (Mimaki et al., 1999; Ichikawa et al., 1997). The saponins display cytostatic activity on leukemia HL-60 cells while the chalcones exhibit estrogenic properties. So far we can find no plant metabolite that could be responsible for the activity observed in the present study. The potency and selectivity of this extract, however, warrants further investigation.

A preliminary examination of extracts from plants used in African ethnomedicine appears to provide some scientific basis for their use in the treatment of serious mental illness. Although previous phytochemical studies of some of these plants may point to the identities of some of the bioactive compounds, no such information is available on other plants. Therefore, in our continuing search for novel neuroactive compounds, further studies will be launched to identify the active principles in these plants. Given the promising beginnings of this venture, we also propose to

expand our investigation to include many more plants in the coming year. Additionally, we intend to include additional assays in the test battery.

Indole Alkaloids and "Mental Illness"

An analysis of plants used in African traditional medicine for the treatment of psychosis showed that many of them contain indole alkaloids. Several plants belonging to the sub-family Plumeroideae (of the Family Apocynaceae) elaborate different mixtures of indole-alkaloids that could account for their application in the preparation of remedies for psychiatric disorders. Indian Ayuverdic medicine documents some of the earliest uses of plants, specifically *Rauwolfia serpentina* (Apocynaceae), for the treatment of mental illness. It would be at least three thousand years before reserpine, a major bioactive constituent of this plant would find its way into Western medicine. Since then ethnopharmacological studies have found a number of other plants that are used by indigenous cultures to treat mental illness. One recurring trend among these plants is the presence of indole alkaloids (see figure below). This class of compounds is of particular interest because of its close structural similarity with serotonin, a neurotransmitter implicated in mood, appetite, sexual function and a myriad of other physiological mechanisms. The aminoethylindole fragment is also found in the structure of melatonin, a hormone responsible for regulating the human circadian rhythm. Therefore, it may be more than mere coincidence that indigenous cultures separated by vast distances have tended to rely on the same class of compounds for the treatment of mental illness. Due to their diverse neuropharmacological effects, plant derived indole alkaloids have generated varying degrees of interest from time to time. A number of these compounds have made significant contributions to modern pharmacology and medicine. However, there has been no systematic investigation that links the neuropharmacological signatures of these compounds with their structures. Such a study could provide a useful road map for the design and development of potentially useful treatments for mental illness. Because the current venture brings together chemistry, ethnopharmacology and molecular pharmacology, it provides an excellent opportunity for initiating studies of this nature. With additional funding, we intend to have suitable quantities of many of these compounds isolated from selected plants. Pharmacological characterization and SAR studies would then follow.

1.8 Phytomedicines:

1.8.1 Development of Methods for the Quantitative Analysis of Herbal Medicinal Products:

Considering the extent of use of herbal remedies and food supplements worldwide, a comprehensive quantitative analysis for monitoring the quality of these products is essential. The aim of the present study is to establish quantitative methods to evaluate the quality of phytomedicine preparations used in the treatment of infectious diseases. Our initial study was on the use of Capillary Electrophoresis in the determination of biflavanones in three Traditional African Medicinal Formulations.

A rapid capillary electrophoresis (CE) method for the quantification of four biologically active biflavanones present in three different traditional African medicinal preparations prepared from the seeds of *Garcinia kola* was developed. The four biflavanones of interest (GBI, GBII, GBI-glycoside and kolaflavonone) were quantified in Hepa-Vital tea, Streptol, and Hangover tonic formulations. The optimum separation conditions consisted of a 100 mM borate, pH 9.5 running buffer, which gave baseline resolution of all three components in less than 15 minutes. Linear calibration ranges for each component were between 5 and 1000 µg/ml. Limits of detection for the biflavanones quantified in this study were between 3 and 6 µg/ml. The "fingerprint" of the

biflavonoids in the aqueous tea and two ethanolic formulations was found to be similar, however concentrations of the four biflavanones was up to 50 fold higher in the ethanolic preparations (tinctures). The major component in all three formulations was GBI.

This study has successfully demonstrated that CE could serve as direct and rapid method for determining the biflavonoids constituents in different herbal formulations containing biflavanones. This method can serve as a means of quality control for regulating raw materials, their extracts and finished products. The unique and powerful capabilities of CE including high resolution and short analysis times, make it a powerful analytical tool in the quality control of above products and other flavonoid-containing products such as *Gingko biloba* proprietary products.

1.8.2. Standardization of Plants used in Traditional Medicine as Herbal Products:

During the current study period, we continued the project on the standardization of plants used in traditional medicine. We produced pharmaceutical-grade dossier of 6 herbal products to aid the local production and regulation of these herbal medicinal products. In addition, the group conducted clinical outcome evaluation of traditional medical practice in the treatment of malaria, leishmaniasis and chronic fatigue syndrome.

1.9 Training:

Samar Benhussein, a premedical student from Catholic University Washington DC, who was interested in medicinal plants; their chemistry and pharmacology, worked at WRAIR utilizing BDCP-ICBG plant samples. The student was taught extraction, chromatographic fractionation and isolation of bioactive compounds from African plants. The student was supported by the Science & Engineering Apprentice Program (SEAP) summer program for a period of 8 weeks. The SEAP summer program is operated in conjunction with Department of Defense (DOD) laboratories and the George Washington University. Its purpose is to give academically talented students, ranging from Middle School through College, a hands-on experience in a scientific laboratory under the guidance of a mentor. The program lasts 8 weeks (8 hours per day - 5 days per week). Students are required to work the entire 8-week period (no vacations) and to produce a poster and a paper to document their work. The program offers students a unique and positive experience in their fields of interest, thus encouraging them to pursue careers in science and engineering.

BDCP provided the student with all the necessary plant materials as well as acting as the student mentor in the area of natural products.

2. BIODIVERSITY CONSERVATION

2.1 SI/MAB – BDCP Training:

Adaptive Management Workshop in Nigeria.

Local implementation of the objectives of the Convention on Biological Diversity in Nigeria, including the crucial aspects of biodiversity monitoring, assessment and inventory (Article 15) has been constrained by the absence of an integrated approach to environmental resource management, broad stakeholder participation, and a lack of coordinated action. It is against this background that SI/MAB and BDCP organized a one-day stakeholder policy workshop on April 18th, 2001, at the Bioresources Development Centre (BioDEC), Abuja, Nigeria. This was the first of a three-phased project that will now be followed by a training course and field assessments. It was organized in collaboration with local partners, including the Federal Ministry of Science and Technology (FMST), the Federal Ministry of Environment (FME) and the National Parks Service (NPS).

The meeting was very successful with a total of 27 participants in attendance from twenty states and four federal government ministries. The participants were comprised entirely of directors of policy-making departments and agencies. This profile significantly enhanced the quality of the discussions and the output of the meeting.

The meeting deliberated on framework strategies for conducting a national multi-taxa biodiversity monitoring program and for integrating this into national programs. By bringing together policy makers from the federal and state government levels, as well as the non-governmental organizations and activating dialogue with the stakeholders, it has successfully laid the foundation for the biodiversity assessment and monitoring course, as well as the follow-up field assessments in the Okwangwo-Gashaka Gumti region. Furthermore, the participants gained a stronger understanding as to the conceptual framework involved for the initiation of a biodiversity assessment and monitoring program.

Results from this workshop have already fed into the follow-up phases of the project and will continue to do so as well as enhance its participatory base. In this regard, separate meetings were held on the 19th of April with officials from FME and NPS to deliberate on the logistics for the course and the follow-up field assessment and the feasibility of the transborder natural resource management concept.

Objectives of the policy framework meeting:

1. Development of a conceptual framework for integrated biodiversity conservation and monitoring program.
2. Identification of modalities for establishment of bioinformatics infrastructure for sustainable use of natural resources in Nigeria.
3. Examination of the role of agrobiodiversity in national food, agriculture and biotechnology policy.
4. Determination of modalities for establishment of a national multi taxa biodiversity monitoring program and database.
5. Examination of methods for linking ethnobotany to drug development and sustainable livelihoods.

Presentations.

The meeting was designed with a focus on dialogue rather than the lecture format. However, the adaptive management framework was presented in a more structured format and formed the basis of the following discussions. The content of the presentation focused on how to develop long-term biodiversity monitoring programs within an adaptive management framework. Additional presentations were made on the ICBG program, the Takamanda project and the socioeconomic valuation and assessment program in Nigeria. Instructors included Chris Ros, SI/MAB, Dr. Maurice Iwu, BDCP, George Chuyong, BDCP-C, and Anthony Onugu, BDCP.

A case study from India on community-based assessment projects was presented by Aparna Watve. Following this, reports from several participants were presented.

National Parks Service:

The NPS was established in 1979 and today has responsibility for eight parks, a number expected to rise to 15. He noted that about 80 percent of designated reserves and community forests were under

the jurisdiction of state administrations and local councils, hence the importance of engagement of state agencies and policy makers. He observed further the crucial challenge before the NPS was in the area of enforcement and capacity building. Although the National Parks law provides for creation of buffer zones, the integrity of designated areas is constantly breached. These issues combine to constrain monitoring and assessment projects. At the same time, there is a need for creation of a common platform that will permit integration of data from the SI/MAB protocol with the existing framework for 1-hectare plots in Nigeria.

Federal Ministry of Environment:

In line with its national mandate, the FME has a number of projects in the pipeline. These include plans for a nationwide wildlife survey, a new national effort to contain invasive species for which an inter-ministerial committee has been set up. Plans are also underway to promote community herbal health centres in 6 states in the North and 9 in the South. Funds will be provided to communities for fencing of such sites, as well as consultants for technical support.

Forestry Research Institute of Nigeria (FRIN):

Current projects are concentrated in the Omu and Ore-Gambari reserves. FRIN is expanding the herbarium in Ibadan and plans to initiate new ones in different ecologies in the country. It has also published works on gully erosion in Enugu. Expressed interest in collaboration on the AMP in order to examine possible adaptation of the SI/MAB protocol in future work. Suggested that necessary contact be established with the Federal Department of Forestry in the Federal Ministry of Agriculture to facilitate additional participation in the course.

Delta State:

Noted that implementation of programmes is severely constrained by a general lack of awareness at the community level. Current projects include the establishment of a medicinal plants garden. Expressed interest in further participation in the AMP but requested for timely notification.

Ekiti State:

Local forestry development program has been initiated with each local council required to designate a fifty-hectare plot for the Forestry Department. A policy of discriminatory felling girths and prices/rates has been introduced into the forestry tariff as a strategy to conserve biodiversity. Forest fire protection strategies and boundary clearing program have equally been initiated. Twenty-five percent of revenue from the forestry sector has been set aside by the state for replenishment of forest reserves under the Forestry Trust Fund Plantation Program. Expressed strong interest in participation, and called for timely notification on the following phases of the AMP.

Edo State:

Collaborating with the FME in providing legal recognition of the "Chief's Forests" in the state, in a manner to merge traditional and modern legal mechanisms for protection of forests. This also includes the gridding and fencing of such reserves.

Follow-up meetings

Federal Ministry of Environment:

A meeting was held with M.M Umar, Director Biodiversity at which the details of the policy meeting was rehashed. It was resolved that the Director will continue to provide oversight functions over the project. It was equally resolved that the FME will serve as lead governmental institution in the implementation of follow-up activities, working through the NPS. The meeting also explored the feasibility of establishing a clearing-house mechanism especially in relationship to a national multi-taxa biodiversity monitoring program.

Federal Ministry of Environment:

Two meetings were held with the responsible ministers: Prof. T. Soun and Pauline Tallen. The meetings were designed to enable buy-in by the ministry. It was resolved that Dr. Omaliko and the BioDEC will remain the contact points for the ministry on the adaptive management project.

National Parks Service:

The meeting was attended by the full complement of the management of the NPS. The Conservator General explained that the principal challenge for the organization lay in capacity building and enforcement of existing regulations. In this regard, he sought for postponement of the course to a more suitable time. It was observed that with regard to the monitoring and assessment plots, the NPS would provide access to the parks for the AMP. However, it was noted that a crucial problem lay in integrating between the existing framework and the SI/MAB protocol. The NPS indicated interest in collaboration on a transboundary project, explaining that a similar one already existed through the Lake Chad National Park. It was resolved that the NPS will provide institutional support for a participant from the CRNP to attend the Smithsonian course in the United States in May. Mr. Musa Wari from the NPS Management Information Unit was appointed as the contact point for future collaboration. It was also resolved that efforts will be made through SI to acquire satellite images of forests from US institutions as well as basic communication and office infrastructure. In addition, the meeting resolved to explore the possibility of publicizing the work of the NPS at the CBD for comments, and collaboration with a pharmaceutical company for a bioprospecting project, with the goal of sustainability and equitable sharing of benefits.

Output of the Meeting:

The meeting resolved to establish a working group for purposes of continuity in execution of the remaining phases of the project. Two principal contacts were appointed, Sha Pam, FME, and Gladys Adams, FMST/BioDEC. Other members were nominated from the Department of Forestry, Forestry Research Institute and two state representatives. It was equally resolved that responsibilities regarding arrangements for the follow-up activities will be devolved to the WG subsequently.

The meeting also resolved to reactivate efforts at the establishment of a clearing-house mechanism, especially against the background of a national multi-taxa biodiversity monitoring program and based on the existing efforts of BioDIN. The meeting also harped on the need for range management and creation of reserves to meet the multiple demand profile of forests. It hoped that this problem would be resolved by the adaptive management framework.

It was also resolved that efforts will be initiated through FRIN and BDCP, with assistance from the Smithsonian for publication of environmental books and leaflets to feed into grade school curricula.

Finally, the meeting determined that there was need for a national conference on biodiversity conservation, inventory and sustainable use.

3. PLANT COLLECTION, ETHNOBOTANICAL STUDIES, AND ECONOMIC VALUATION:

3.1 Specific Aim:

- a. To conduct ethnobotanical inventory of plants in the selected study areas.
- b. To maintain and expand the database on African medicinal plants
- c. To guide the ICBG in its plant selection and collection strategies for drug discovery.
- d. To conduct a socio-economic value assessment of the biological resources in the study area.
- e. Assist in capacity building of West and Central African scientists in the area of ethnobiology, inventory, field taxonomy, economic value assessment and research management.

3.2 Progress Report:

During this project-reporting period (September, 2000-July, 2001), we continued the ethnobotanical studies and socio-economic evaluation of plant species in areas not yet covered. With the support of CARPE, we also evaluated and published a report on the use of phytomedicines as an economic incentive for biodiversity conservation in Cameroon.

3.3 Ethnobotanical studies:

We are in the process of analyzing the data collected and publishing the report in a usable CD format. Upon completion of this report, the electronic versions will be available to collaborating institutions both in Africa and the United States. The Nigerian ethnobotanical studies, which cover a more heterogeneous population than Cameroon, have been continued with greater emphasis on the Niger Delta region of the study site.

3.4 Plant Collection:

In collaboration with AP-1, the protocol for random collection from both the large plot in Cameroon and the smaller 1 ha plots in Nigeria and Cameroon has been finalized. Using a computerized random selection, we have selected 600 plants for random sampling. These will be screened in the cytotoxicity, antimalarial, brine shrimp and cystic fibrosis screens. Random collection success rates will be compared to the ethnomedical collections for a number of screens. Target species for drug development from our ethnobotanical and preliminary studies are also being collected in large quantities to enable AP-2 isolate sufficient quantities of compound for *in vivo* studies.

3.5 Socio-economic Value Assessment

3.5.1 Summary

The management of natural resources has emerged as an issue of strategic policy and scientific import for economic development planners, conservationists and resource users. At the same time, it has been said that one of the major constraining factors in the conservation, sustainable use and management of natural resources is the failure to assign proper value to the functions and services of natural ecosystems. According to the report of the subsidiary Body on Scientific Technical and Technological Advice (SBSTTA) of the Convention on Biological Diversity, failure to properly value natural resources sends wrong signals to decision makers, and misleading information about their abundance, thus providing inadequate incentives for the management, efficient utilization and enhancement of biological resources.

The main objective of the Economic Valuation Studies component of the ICBG is to address the problem of apparent inability of local users and policy makers to recognize or commensurately value the functions and services of tropical forests that has for long constrained conservation efforts. As a result of the improper evaluation of forest resources, the total economic value of forest resources as reflected in official documents and publications rarely influence decisions bordering on their exploitation and management. The underlining aim of the ICBG project is to provide an economic framework for the efficient use and sustainable management of natural resources. Although historically indigenous populations have exploited non-timber forest products (NTFPs) from tropical forests, greater attention has been given to wood and wood products. However, there is now a growing tendency to acknowledge the total value of natural ecosystems, including tropical forests.

The role of economic valuation becomes especially important as a mechanism for capturing and assigning total economic value to natural resources. Through this, it is then possible to show that NTFPs also matter for planning at the microeconomic level, and to demonstrate its necessity in making efficient allocative decisions at the macroeconomic level.

The Study Area:

In Cameroon the project is based in the North West Province (N.W.P) and the South Provinces (Southeast, Southwest, Western and Central/ Atlantic). From the borders in the southeast, southwest and on the Atlantic coast, Cameroon is covered with thick green forests, intersected by large rivers. Although there has been frequent migration to and interaction with cities, the villages have kept their traditional stamp. As a startling contrast to the rough vegetation of the south, the landscape of North Cameroon is Savannah grass. The N.W.P. has an area of about 17,409 km² lying between lat. 5°43' north of the equator and longitude 9°12' east of the Green Meridian. It is bounded on the west and north by Federal Republic of Nigeria, to the south by South West Province, to the southeast by the Western Province and to the far east by the Adamawa Province. The province is divided into seven administrative units called divisions. Each of these divisions is made up of seven tribes whose cultural backgrounds portray assorted and unique ways of utilizing the vegetation. Our choice of study areas took cognizance of this fact.

In Nigeria, two local government areas in Imo and Ebonyi states were sampled. The choice of the two states was deliberately made to reflect areas of this region of Nigeria of a semi-urban nature in order to capture the dynamics of a predominantly natural resource based economy as exists in rural areas of the country, side by side with commercial influences from the urban areas.

Economic Valuation Methodology:

It is widely recognized that there is no single definition of value, and that definitions are adopted on the factor of the particular purpose for which valuation is required. In the case of genetic resources, determination of value has been largely polarized by the divergent concepts of natural ecosystem values between economists and ecologists.

In economic theory, the assignment of value to a given resource is among other things, based on its possession of (i) Utility-ability to satisfy desires or create goods or services (ii) Capability for ownership and (iii) Limitation in supply. For economists, the objective value of goods and services is that which is determined by market forces. And for non-market goods, imputed prices are used to establish value. Conversely, the ecologist will add to the above, the intangible value inherent in the ecosystems performance of a free service to man.

Conflicts and ambiguities have arisen from misinterpretations of these perspectives (Farnworth, Tiddrick Jordan, and Smathers, 1998). Yet these perspectives are important to a proper understanding of the value of natural resources. A more conventional approach, which combines the two perspectives, is now being adopted. This categorizes value into use or instrumental value and non-use or intrinsic value.

Use Value describes the ability to satisfy wants or desires directly or indirectly. While the direct use value may be easy to conceptualize, it is not necessarily easy to measure in economic terms especially in the case of medicinal plants. On the other hand, indirect values refer to the ecologists' concept of the value of the watershed function of ecosystems.

Option Values relate to future use of natural resources being an amount that individuals are willing to pay to conserve it for future use. There is also the Quasi-Option Value, which refers to the value of learning about the benefits that would be precluded if development rather than conservation of given natural resources were chosen now.

Non-Use or Intrinsic Value refers to the value associated with knowledge of an environmental asset as independent of its current or future use. However, a divergence of opinion has sometime occurred as to whether value can be assigned to both conscious (material) and unconscious (spiritual) objects or whether only conscious or material objects can possess intrinsic value (Regan, 1981).

Total Economic Value: The concept of total economic value is important to the assessment of the value of the natural resources of developing countries. This will enable these countries to capture the real value of their biological resources for the benefit of their communities, nations and the world at large.

The basic approach here is to determine the overall value of natural resources to various stakeholders in the community. In other words, the true value of this to an individual from the moment he wakes up in the morning to the time he retires to sleep. In local communities in Nigeria and Cameroon, the use value of natural resources is concentrated on, and varies across individuals and stakeholder groups. On the other hand, Non-Use or Intrinsic values largely reflect community value of natural resources. This includes resources specifically designated as communal, sacred groves, even where dedicated to a specific deity, and indigenous knowledge.

It is our view that the total value of natural resources should reflect these. It follows therefore that assessment techniques should reflect the following considerations:

1. They should be participatory in nature.
2. They should involve household and socioeconomic surveys so as to determine the structure of user groups and the spatial variation in values across user groups, product availability, market structure, pricing etc.
3. They should involve assessment of NTFPs and their associated off-farm income generating activities.
4. Finally, they should involve assessment of the institutional capabilities of indigenous social groups to assume new roles in resource management.

The determination of the economic value of natural resources for pharmaceutical drug discovery is therefore an important but certainly not the sole criteria for assessing the value of natural resources. It follows therefore that the missing link in establishing an equitable economic incentive for both biodiversity conservation, combating of desertification and sustainable use of natural resources, is a mechanism for internalizing the external benefits and cost associated with the use of genetic resources.

Contingent Valuation studies serve several functions. While the focus of the EVS has been on the resulting values, contingent valuation uncovers considerable amounts of information about what local people want. It also serves as a vehicle for public participation. At a time when it is now recognized that many investments 'fail' because of a lack of consultation and assessment of local wants and needs, this role for survey techniques should be emphasized.

Factors affecting value

The concept of the value of African natural resources is influenced by two contending views on appropriate mechanisms for conserving biological resources and dryland development. In the first view, rapid economic development and industrialization is seen to increase the pressure on arable land leading inevitably to environmental degradation through intensive agriculture, opening up of forest land and over exploitation of forest resources.

In this case, the conservation policies adopted often range from the designation of areas as protected to restrict access to it, afforestation of drylands with fast growing species, to the imposition of carbon tax. Natural resource values are thus largely seen, from the perspectives of the willingness to pay and willingness to accept compensation logic.

The second view begins with recognition of the role of poverty in environmental degradation. Natural resource conservation is thus seen as one facet in the nexus of economic development of indigenous communities. It establishes that a link exists between vigorous indigenous culture and the preservation of natural resources by various stakeholders in the community, and the value they place on these resources as against some esoteric value from outside (Iwu, 1996)

Data on specific forest products were generated with respect to product type, usual period of collection, quantity and type of product. The revenue generated from harvesting medicinal plants was established by using primary data obtained in the household survey. Specifically, information was sought with respect to the species, plant part collected, period of collection, frequency of collection, quantity, price and market sold. Finally, contingent valuation methods (CVM) were used to determine willingness to accept compensation from government for loss of a community forest, and willingness to accept compensation for loss of a medicinal plant.

3.5.2 Results and Discussions

To assess the total value of non-timber forest resources to indigenous communities, survey data was obtained from a sample of households in two local government areas of Imo and Ebonyi states of Nigeria, as well as in Oku, Sabga and Bafut in Cameroon. A total of 300 households living on the fringes of forests and relying on forest resources for most of their consumption goods were sampled.

The survey evaluates the exploitation and uses of various plant species and other forest products in the communities sampled. It assesses the knowledge levels of forest users concerning medicinal

plants and other forest products, and determines the use and non-use value of plant species using both the price mechanism and contingent valuation techniques.

i. Non-Timber Forest Products of Natural Ecosystems

Non-timber forest products (NTFPs) are biological resources other than timber, which are harvested either from natural or managed forests (Peters, 1994), and grasslands. Because of their number, resource richness, end-use variation and versatility, they represent a challenging product group and important sources of revenue. In Indonesia for example, exports of NTFP rose from \$13 million in 1973 to \$154 million in 1985 comprising 12 percent of the export earnings of forest products (Pearce, Barbier and Markandya, 1990). Examples of NTFPs include fruits, nuts, oil seeds, latexes, resins, gums, medicinal plants, spices, wildlife and wildlife products, ornamental plants, and raw materials such as bamboo and rattan.

Historically, indigenous populations have exploited natural ecosystems through the use of non-timber forest products for daily sustenance as sources of food, medicine and materials for shelter. However, economic assessment of the value of natural resources has been skewed in favor of timber and timber products to the detriment of NTFPs and the off-farm income generating activities, which they spurn in rural households. This has been attributed to the parameters utilized for measurements, which emphasize 'commercial significance' of forest products, which are subject to international trade (Poulsen, 1982).

Our studies have shown that while NTFPs may be outside statistics on official commerce, they could contribute to savings in foreign exchange. For instance, shea butter from *Butyrospermum parkii* tree can be an alternative to the importation of cooking oil (Poulsen, 1982). The Madagascar flora is said to comprise some 400 species of aromatic plants, but only a fraction of this is reflected in commerce (Rajaonarivony, 1996). The reliance of a majority of people in the developing world on NTFPs as herbal medicines is well known and documented (Falconer, 1990). Our studies in rural Nigeria have revealed the existence and use of several medicinal plants for treatment of various ailments. The widespread use and high knowledge levels of medicinal plants as compared to the relatively low cash income realized from its sale as revealed by the study, reflect an indigenous value structure that is not adequately captured by market values (Onugu, 1999).

Recognition of this value of NTFPs could provide the missing link in the proper and full understanding of the African environment, its value and its use as a tool in the social economic development of the continent.

ii. Off-Farm Income Generating Enterprises from NTFPs:

NTFPs provide a wide range of raw materials and inputs for a diverse array of rural enterprises. In the process it provides off-farm employment to large segments of the rural population, especially in the off-farm season.

These products are largely derived from extracts of plants, including fruits and seeds, and vegetative structures such as stems and barks. They include the production of alcoholic beverages, wild honey, soaps, mats, caps, beds, brooms, baskets, cups, gourds, sponge, fish nets, cane chairs, ropes, ornaments etc. In the case of cane chairs, production systems have advanced and attempts have been made to export the finished products. In the production regions, guilds or trade groups have developed from this enterprise to regulate exploitation of cane, enforce standards and protect the interest of members. Finished products such as mats, brooms, baskets and ropes have become important products in the savanna region of Nigeria for storage and packaging of agricultural goods

for sale in distant markets. The demand for these products has become so high as to necessitate supply from the southern region. There are indications that these enterprises possess features that are amenable to further development. Further study is required in this area to improve knowledge of secondary products of NTFPs, the extent of value added, employment generation and impact on both household and local income.

Table 8: Products from forest-based enterprises

Product type	Local name	Plant material made from	Price/Unit	No. of Units/year	Rev. in 1996
Mat	Ute	Raffia fronds	-	-	-
Cap	Okpu	Palm	-	-	-
Broom	Aziza	Palm frond	9	85	642
Bed	Akpakara	Bamboo	1705	-	68050
Baskets	Nkata	Palm frond	20	98	2075
Fish net	Nzara	Raffia palm	-	-	-
Sponge	Sapo	-	-	-	500
Cane chair	-	Cane plant	-	-	87725
Rope	-	Climbing plants Palm fronds	-	-	4500
Raffia	Agwo	Raffia palm	100	-	12000
Honey	-	Honey bee	-	-	-
Bamboo stick	Achara	Bamboo plants	350	44	16200
Walking stick	Nkpa	Forest plants	-	-	-

Source: Field Data, 1996.

iii. Country Surveys:

a) Southeastern Nigeria:

Household Profile

The household sample comprised of 248 males and 52 females. Majority of the respondents were in the age bracket of 41-50 years. Most of the survey population had no formal education (76%).

As is typical of occupational distribution patterns in rural areas of Nigeria, 75% of the respondents report farming as their primary occupation, while 36% regarded it as secondary. Other major occupations were traditional herbal practice, wine tapping, civil service and training. The average household head reported total annual earnings of N22,942 and N15,280 from major and minor occupations, respectively (100 Nigerian Naira(N) = USD1).

Exploitation and Use of NTFPs

The survey revealed that respondents had free access to community forests, many of which were primary forests, with about 75% of the sample living an average of 3km from a major forest area. The use values structure of forests in the survey show a great diversity of functions to indigenous people in the communities sampled. Of highest significance to the respondents is the perception that forests provide the habitat for biodiversity, which are considered to have medicinal value. Following closely in importance is farming and other economic activities, which take place in forest areas. Allied to these is the value of forests as a source of food and other forest products such as fruit, nuts, latex, honey, rattan, meat and oils.

The importance of the forest is also reflected in the respondents' ratings of their knowledge levels of plants, especially medicinal plants, high as indicated by about 47% of those surveyed.

Using the price mechanism and direct market values, the study estimated income from food and medicinal plants. Except in a few cases, the data shows a nominal increase in the amount realized from sale of forest products by users. As household incomes become eroded by unfavorable exchange rates, the economic importance of forests and their products relative to agriculture and allied activities begin to rise. This is expected to have negative implications for conservation and sustainable use of biodiversity.

Non-Market Value of Plant Species:

a. Willingness to Accept Compensation Estimates: An empirical effort is made in this section to measure the value of forest resources, which are not exchanged in the market in order to determine their non-use values. Having first established the preference of the surveyed for such non-marketed plant species, the CVM was employed to determine the willingness to accept (WTA) compensation with respect to medicinal plants.

The WTA estimates per unit of medicinal plant species was found to range from as low as N20 to as high as N8000. The average estimate of WTA was N2471.92. This was far less than the average for traditional herbalists (N3793.75), which reflects the greater use value from that occupational group. The estimates also reflect a greater value attachment to forest resources with multiple uses.

b. Willingness to Pay (WTP) Estimate: Respondents were asked how much they were willing to pay as compensation for specific forest areas on the one hand, and specific NTFPs on the other hand. The WTP estimates in respect of specific forests in the communities investigated ranged from N3000 to N6.5million.

Table 9: WTP Estimates for Specific forests

Community Forests	WTP (N)
Umuoyim	10200
Mbaraocha	88000
Ofeiyi	6000
Ohia Emedo	3000
Ikpa-laika	5000
Oke-Oha	4000
Okeohia Umuze	16000
Ekpezize	84000
Okeohia- ofukpa	30000
Epenwaopara	20000
Ofeikpa	20000
Epe Ikpem	60000
Epe imo	30000
Onuagu	22000
Aleke	22000
Okeohia-osineke	112000
Alajerya	99000
Ude Otukpo	200000

Ajaohia Umuduruokoro	6.5m
Okeohia Unudimgo	2.5m
Community Forests	WTP (N)
Ajaohia Okoronkwo	22000
Osisifiyifiyo	800000
Owereukwu	250000
Agambo ogara	100000
Ekwe okwu	100000
Total	11303200
Ajaohia Ibeabuehi	200000

N=Nigerian currency (Naira)

The WTP estimates in respect of specific NTFPs were smaller ranging from N100 to N0.5million, with a mean value of N30,278.79, which by implication is the amount an average user of forest products would be willing to pay for its protection.

c. Willingness to Accept (WTA) Estimate as compensation for Forest Degradation: WTA estimates were also derived from respondents for compensation for forest area degradation and destruction of specific NTFPs, and ranged from N300 to N1.69million. The WTP values for specific forest areas were found to range between N22,000 and N7million, a mean value of N1.5million. The discrepancy in both figures can be attributed to the explanation that people are less willing to spend actual income or wealth as opposed to "opportunity" income or wealth (Knetsch and Sinden, 1984).

b) S.W. Province, Cameroon

Exploitation and Use of Non-Timber Forest Products:

Information collected shows a wide degree of access to and use of community forests in food consumption and its importance in the diet, plant medicines and for house building, household and agricultural equipment, fire wood, fodder and in trade and processing activities. Consumer surveys were undertaken to assess the demand for bamboo, cane and raffia palm products etc and the extent to which people rely on herbal medicines.

Medicinal Uses:

Data from the BDCP small plots is dominated by forest medicines. These are highly valued as sources of natural medicines, which are essential components of health treatments throughout the region. They are the main medicines used by a vast majority of all classes of people and despite the many different healing practices - they are still commonly used in conjunction with mystical and ritual practices. The sacred value attached to some plants like *Adenia lobata*, Warrior liana and royal reserves confirms the link between healing and spiritual values.

Survey results from local communities testify that about 70% of the sample population relies on wild medicinal plants as main source of treatments. The general belief is that certain diseases can best be treated by traditional plant cures notably epilepsy, mental disorders and spiritual problems. It was noticed that the use of herbal medicines is not restricted to specialists. By far the most common users of medicinal plants as self-administered first aid are the women who play a central role in the first aid treatments of their children. This indigenous knowledge is passed on in families and even young ones are well versed in the use of medicinal plants as first line health care product.

Most herbal medicines encountered were given both as curative and preventive. The latter were often added to soup and used as blood tonics or to increase the lactation of a newly delivered mother. Others are taken as infusions, or decoction. It was definite that the medicinal uses of forest plants is widely known as some trees were left on farms and back gardens because of their medicinal properties.

Non-Medicinal Uses:

Tropical forests provide essential raw materials and inputs that support assorted rural enterprises and provide employment to large segments of the rural population. Extractives such as oils and wines are common products. Many of the finished products from forest-based enterprises have considerable values in both local and international markets, and cover such areas as local crafts manufacture, processing and artisans.

These enterprises exist in an informal but organized sector, according to nature of activity, producers, processors and marketers. They thus present an unused potential for exploitation in biodiversity conservation.

From the 547 plants collected through household questionnaires it was certain that forest contribute to all aspects of rural life providing bush foods fodder fuel, building material, household items as well as many intangible benefits such as the cultural and symbolic (figurines and decorative artifacts). Each community is unique in its type of building materials due to the differences in the availability of the plants in their environment.

Although forests maintain environmental stability as well as sources of income, they furnish bush food for animals and humans. This bush food is consumed occasionally by women and children who use leaves for food wrapping, wild spices, as well as scientist and hunters searching for game and medicinal plants. Many bush foods are either flavourants (*Xylopia* sp.; *Xymalos monospora*; *Piper guineense*; *Piper capense*; *Aframomum melegueta*; *Aframomum pruriens*; and *Afrotyrax lepidophyllus* or wild fruits of *Aframomum danielli*; *Annona senegalensis* etc. Mushroom gathering was witnessed as a serious hobby and only three species were collected - *Termitomyces striatus*, *Agaricus* sp. and *Polyporus* sp. Collectively these flavourants add diversity and flavor to the diet as well as provide proteins, energy, vitamins and minerals. They are of particular importance to patients suffering from blood wasting diseases and lactating mothers who compared to adults consume greater quantities of food, to acquire additional vitamins when they are most vulnerable to anemia.

Furthermore there is widespread use of items made from NTFPs in daily life, but the range of the goods varies within and between the tribes. The highly valued household item in most villages is the pestle made from *Raphia vinifera* and *Harungana madagascariensis*. Survey results from BDCP projects show that it was ranked as the most important item above all other forest products. Formerly pestles used to be made also from *Pygeum africanum* but with the discovery of an active ingredient for the treatment of benign prostatic hyperplasia (BPH) from it, over harvesting of its bark has limited its use.

Other essential items in the kitchen include spoons, grinders, baskets, mats and chairs. Forest resources are also widely used for making musical instruments, carved tools, building of bridges, pit latrines, canoes, sleeping and fencing mats, as well as utensils, sponges, brooms, and sandpaper. They also supply material for agricultural equipment such as hoe, axe and cutlass handles, yam stakes etc.

One of the most exhaustible plants observed was the cane *Ariendinaria alpina* that is almost extinct. The trade in this climbing plant is enormous and there are no campaigns for its replacement.

Leaves from several species are widely used by traders and food sellers as packaging material. The leaves most commonly used are from the musaceae and marantaceae families. The former is collected from around houses and the other from swampy sites. The former must be warmed over a flame to prevent it from tearing. *Marantaceae* leaves are strong, durable, impermeable and able to withstand heat, all of which makes them invaluable to food sellers and traders. These leaves are collected regularly by women for the wrapping of fish, salt, meat, and cooked food notably pounded cocoyams. They also impart flavor to the cooked food as well as its preserving qualities. It is conceivable that these natural packaging materials will find universal appeal among natural product conscious consumers.

The Role of Women:

The involvement of women is limited as some traditions restrict them from touching certain plants like *Ceiba pendrandra* (Silk cotton), *Dracaena deisteliana* (Peace plant) or from uprooting cultural plants - *Raphia vinifera* (Raffia palm), *Elaeis guineensis* (oil palm) and *Cola acuminata* (Kola nuts). This is especially the case during the menstruation cycle. In spite of this, the gender distribution of roles shows that women play an important role as food providers for their families. They are responsible for firewood and first aid treatments for their children, as they are the first to diagnose and treat their children. Evidence from BDCP projects shows that women have an intimate relationship with the forest. This is reflected in their unique vocabulary and diversified knowledge of all sorts of plants. This is further enhanced through wood fetching, farming, and primary health care experiences of their children's health. In large part, they can be said to be the custodians of scarce and lost crops of Nigeria and the Cameroons. Their knowledge of herbs for fertility disease is unique.

Apart from these restrictions, most women's income are largely earned from the sales of wild spices food wrappers, food crops and vegetables.

Marketing of NTFPs:

Through the household surveys, the following plants have been identified in the market for non-timber forest products in Nigeria:

Table 10: Common Plant Species and their uses

Plants	Family	Type	Uses	How used	Market sold
<i>Dacryodis edulis</i> Igbo: Ube	Burscraceae	Fruit	Food	FC	Local
<i>Treculia africana</i> Igbo: Ukwá	Moraceae	Fruit	Food	PC	Local
<i>Pentaclethra macrophylla</i> Igbo: Ugba/Ugbakala	Caesalpiniaceae	Fruit	Food	PC	Local
<i>Cola nitida</i> Igbo: Oji	Sterculiaceae	Fruit	Stimulant	F	Local
<i>Garcinia kola</i>	Guttiferae	Fruit	Medicine	F	Local

Igbo: Akilu/Akirilu					
<i>Mangifera indica</i> (Mango)	Anacardiaceae	Fruit	Food	F	Local
<i>Chrysophyllum albidum</i>	Sapotaceae	Fruit	Food	F	Local
Igbo: Udara					
<i>Irvingia gabonensis</i>	Irvingiaceae	Fruit	Food	FC	Local
Igbo: Ugiri					
<i>Anacardium occidentale</i>	Anacardiaceae	Fruit/Nut	Food/Industrial	F	Local
<i>Musa sapientum</i> (Banana)	Musaceae	Fruit	Food	F	Local
<i>Elaeis guinensis</i>	Palmae	Fruit/Oil	Oil	PC	Local
Igbo: Nkwu					
<i>Dennettia tripetala</i>	Annonaceae	Fruit	Medicine	F	Local
<i>Hevea brasiliensis</i> (Rubber)	Euphorbiaceae	Fruit/latex	Latex	P	Distant
<i>Diallum guineense</i> (Icheku)	Caesalpiniaceae	Fruit	Food/ staking	F	Local
<i>Gongronema latifolium</i>	Asclepiadaceae	Vegetable	Food/ medicine	FC	Local
Igbo: Utazi					
<i>Piper guineense</i>	Piperaceae	Vegetable	Food/ medicine	FC	Local
Igbo: Uziza					
<i>Gnetum africana</i>	Gnetaceae	Vegetable	Food	FC	Local
Igbo: Okazi					
<i>Naudea sp.</i>	Rubiaceae	Fruit	Food/ medicine	F	Local
<i>Marantochloa leucantha</i>	Marantaceae	-	Mat	P	Distant
Igbo: Ute					
<i>Megaphrynium macrophyllum</i>	Marantaceae	Vegetable	Wrapping	F	Distant
Igbo: Etere					
<i>Strophanthus hispidus</i>	Apocynaceae	-	-	-	-
Igbo: Osi aguru					
<i>Aframomum melegueta</i>	Zingiberaceae	Fruit	Stimulant/ medicine	F	-
Igbo: Ose oji					
<i>Pterocarpus sayauxii</i>	Papilionaceae	Vegetable	Food	F	-
Igbo: Oha					
<i>Nauclea sp.</i>		Herb	Medicine	P	-
Igbo: Ubulu					
<i>Xylopia aethiopica</i>	Annonaceae	Fruit/Veg.	Medicine	C	Local
Igbo: Uda					
<i>Psidium guajava</i> (Guava)	Myrtaceae	Fruit	Food	F	Local

Key:

F- Used Fresh

C- Used Cooked

P- Processed

Commercial sales of medicinal plants in Cameroon is dominated by Plantecam, a subsidiary of the French company Fournier Laboratories, under exclusivity trade and supply arrangements with European pharmaceutical and natural products companies. In addition, Plantecam operations in

eligibility for national taxes or customs duty. Plantecam/Fournier Laboratories until its winding-up, was wholly French-owned, and did not have Cameroonian shareholders. Annual turnover in 1997 was 1.5 – 2 billion CFA (Sunderland et al, 1997). Lowe et al. (1998) have found the centre of genetic diversity for each *Irvingia* species, which for *I. gabonensis* is around Ebolowa in Southern Cameroon and for *I. wombolu*, is in South-east Cameroon and Western Nigeria.

3.7 Data Management - CISAMAP

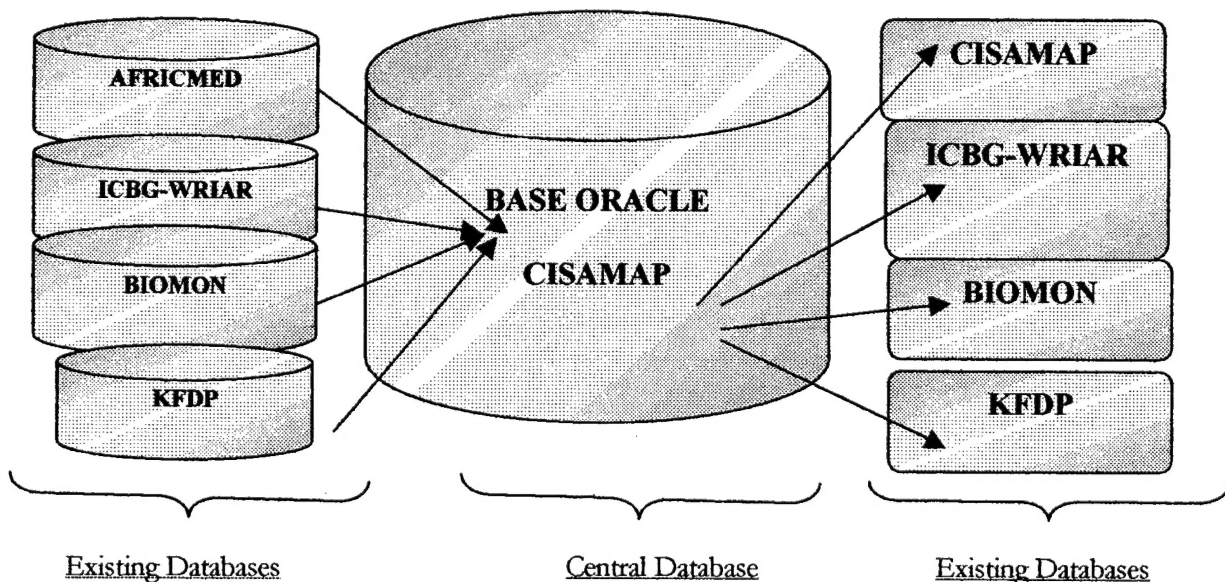
The main object of CISAMAP is to provide a common database that is user friendly and easily accessible. This data should interface with the four existing databases thereby allowing users to access multi-layered information. To achieve this, a determination was made to use oracle software to provide easy conversion to other platforms. The database will provide information on plant taxonomy/ nomenclature, ethnomedicinal uses of plants, chemistry and biological assays as well as biodiversity conservation information and publications. The structure is general to allow any user working on the same theme to find all relevant information. We have thus considered and embodied information related to:

- i. Medicinal plants (here stands for a medicinal plant or an animal since part of animal are also used in herbal medicine),
- ii. Potions or herbal medicines (natural medicine),
- iii. Diseases as represented by traditional healers that treat them.

This general conception is to increase the range of users of the system. Any user interested in only one aspect or a subset of an aspect will receive a projection (view) of the database containing the relevant information. For example, the schema of the database CISMAP shown below is included entirely in our general structure. They will receive a view with the information they are interested in. The used interfaces and the reports will vary accordingly.

In this section, we present the architecture of CISMAP database. Other sections describe the structure of the overall database. The reader can verify that it does contain the information he/she is interested in.

Database Architecture: We have unified the current four databases in one ORACLE database called CISAMAP. A user can choose to work with the whole database, or just one of its subset. If he is interested only in the information related to medicinal plants, the view ICBG-WRIAR will be projected. For someone desiring only information related to chemical and biological aspect of the plant, the view BIOMON will be projected. The view KFDP will be projected if only demographic or geographical information is needed.



3.8 Sustainable Development

The socio-economic evaluation studies will be continued in five more communities. Our ICBG structured questionnaires will be used to elicit information on household characteristics; products/plant species from community forests; revenue generated from forest products; gender issues in respect to access to and use of forest products; knowledge of plant species; marketable and non-marketable values of plant species. Field data from these locations will be integrated into the Computerized Information System on African Medicinal and Aromatic Plants (CISAMAP). A training course on economic evaluation is also planned for next year.

Training courses in Biodiversity measuring and monitoring will be held in Nigeria in October 2001. This interactive workshop will explore SI/MAB permanent plot methodology for measuring and monitoring biodiversity and its use as a foundation for a regional network of biodiversity sites. Using the tested SI/MAB protocol, participants will learn to establish permanent vegetation plots quickly and easily and to analyze and communicate the results soon after the data are collected. The plot information will form the foundation upon which a variety of other monitoring protocols for other taxa can be incorporated into a Geographical Information System (GIS). As in earlier training courses, the focus will be on practical and standardized technique used for monitoring the biodiversity of different groups of organisms, for developing and maintaining databases.

This ICBG will also continue its capacity building efforts in BDCP, University of Dschang, University of Jos and National Union of Herbal Medical Practitioners (Nigeria and Cameroon).

4. REFERENCES:

1. Acharya, Keya. 1998. Prostate Medical Demand Strips Curative Trees. The Environment News Service, March 13, 1998.
2. Acworth, J., BN Ewusi. 1999. *Prunus africana*: Striving for sustainable and equitable resource management in Cameroon. Medicinal Plant Conservation, Vol. 5. IUCN MPSG.
3. Ainge, L. and Nick, Brown "Irvingia Gabonensis and Irvingia Wombolu: A State of Knowledge Report", The Central African Regional Program for the Environment. http://carpe.umd.edu/products/PDF_files/Report-AingeBrown2001.pdf
4. Ames, G. F. L., Mimura C. S., Shyamala V., *FEMS. Microbiol. Rev* 75, 429 (1990).
- Anderson M. P., Gregory R. J., Thompson S., Souza D. W., Paul S., et al, *Science* 253, 202 (1991).
5. Anderson M. P., Berger H. A., Rich D. P., Gregory R. J., Smith A. E., et al, *Cell* 67, 775 (1991).
6. Bacchi, C.J., Brun, R., Croft, S.L., Alicea, K., Bühler, Y. 1996. *In vivo* Trypanocidal activities of new S-adenosylmethionine decarboxylase inhibitors. 40(6): 1448-1453.
- Bear C. E., Li C., Kartner N., Bridges R. J., Jensen T. J., et al, *Cell* 68, 809 (1992).
7. Carson M. R., Travis S. M., Welsh M. J., *J Biol Chem* 270, 1711 (1995).
8. Chen, H., Boyle, T.J., Malim, M.H., Cullen, B.R., Lyster, H.K. Derivation of a biologically contained replication system for human immunodeficiency virus type 1. *Proc. Natl. Acad. Sci. USA* (1992) 89: 7678-7682
9. Cheng S. H., Rich D. P., Marshall J., Gregory R. J., Welsh M. J., et al, *Cell* 66, 1027 (1991).
10. Cheng S. H., Gregory R. J., Amara J. F., Rich D. P., Anderson M., et al, in *Current Topics in Cystic Fibrosis*, J. Dodge, D. J. Brock and J. M. Widdicombe, Eds. (John Wiley, Chichester, England, 1992)
11. Collins, F. S. 1992. *Science* 256: 774-779
12. Denning G. M., Ostedgaard L. S., Welsh M. J., *J Cell Biol* 118, 551 (1992).

13. Denning G. M., Anderson M. P., Amara J., Marshall J., Smith A. E., et al, *Nature* 358, 761 (1992).
14. Desjardins RE, Canfield CJ, Haynes JD, Chulay JD. Quantitative assessment of antimalarial activity in vitro by a semi-automated microdilution Technique, *Antimicrob Agents Chemother* 1979 Dec; 16(6): 710-8.
15. Ejiofor, M. A. N. 1987. Developing improved methods of processing and utilization of kernels of *Irvingia gabonensis* (var. *gabonensis* and var. *excelsa*). *The International Tree Crops Journal* 4: pp.283-290.
16. Falconer, J. 1990. "The Major Significance of "minor" forest products: The local use and value of forests in the West African humid forest zone". *Community Forest Note* 6, FAO, Rome.
17. Farnsworth, E.G., T.H. Tidrick, C.F. Jordan and W.M. Smathers. 1981. "The Value of Natural Ecosystems: An Economic and Ecological Framework". *Environmental Conservation* 8 (4): 275-282.
18. Fields, S. and Song, O., *Nature* 340:245-247
19. Gregory R. J., Rich D. P., Cheng S. H., Souza D. W., Paul S., et al, *Mol Cell Biol* 11, 3886 (1991).
20. Hayashi T, Asano S, Mizutani M, Takeguchi N, Kojima T, Okamura K, Morita, Scopadulciol, an inhibitor of gastric H⁺, K(+) -ATPase from *Scoparia dulcis*, and its structure-activity relationships. *J Nat Prod* 1991 May-Jun; 54(3): 802-9
21. Hirumi, H., Hirumi, K. 1989. Continuous cultivation of *Trypanosoma brucei* bloodstream forms in medium containing a low concentration of serum protein without feeder layer cells. *J. Parasitol.* 75:985-989.
22. Hyde S. C., P. Emsley, M. J. Hartshorn, M. M. Mimmack, U. Gileadi, et al, *Nature* 346, 362 (1990).
23. Iwu, M.M. 1996. "Development of Indigenous Genetic Resources: The BDCP Experience in West and Central Africa". In: *Science in Africa: Utilizing Africa's Genetic Affluence through Natural Products Research and Development*. AAAS Symposium. Washington, D.C. AAAS Sub-Saharan Africa Program.
24. Ito Y and Ma Y., pH-zone-refining countercurrent chromatography. *J Chromatogr A* 1996 Nov 8;753(1):1-36
25. Kartner N., Hanrahan J. W., Jensen T. J., Naismith, A. Sun L. S., et al, *Cell* 64, 681 (1991).
26. Kartner N., Augustinas O., Jensen T. J., Naismith A. L., Riordan J. R., *Nature Genetics Nat Genet* 1, 321 (1992).
27. Kerem B-S., Rommens J. M., Buchanan J. A., Markiewicz D., Cox T. K., et al, *Science* 245, 1073 (1989).
28. Kiser, R., Makovsky, S., Terpening, S.J., Laing, N., Clanton, D.J. Assessment of a cytoprotection for the discovery and evaluation of anti-human immunodeficiency virus compounds utilizing a genetically impaired virus. *J. Virol. Methods* (1996) 58:99-109
29. Knetsch, J.L. and J.A. Sinden, 1984, "Willingness to Pay and Compensation Demanded: Experimental Evidence of an Unexpected Disparity in Measures of Value", *Quarterly Journal of Economics*, 99(3), 507-521.
30. Kuchler K., Thorner J., *Proc Natl Acad Sci. U. S. A.* 89, 2302 (1992).
31. Lukacs G. L., Mohamed A., KartneN. r, Chang X. B., Riordan J. R., et al, *EMBO J* 13, 6076 (1994).
32. Li C., Ramjeesingh M., Wang W., Garami E., Hewryk M., et al, *J Biol Chem* 271, 28463 (1997).
33. McGrath J. P., Varshavsky A., *Nature* 340, 400 (1989).
34. Meingassner, J.G., Mieth, H., Czok, R., Lindmark, D.G., Müller, M. 1978. Assay conditions and the demonstration of nitroimidazole resistance in *Tritrichomonas foetus*. *Anitmicrob. Agents Chemother.* 13:1-13.

35. Milhous, W.K., Weatherley, N.F., Bowdre, J.H., and Desjardins, R.E., 1985, *In vitro* activities and mechanisms of resistance to antifol antimalarial drugs, *Antimicrob. Agents Chemother.* 27:525.
36. Okafor, J.C. 1991. "Improving edible species of forest products. *Unasyuva*, 165 (42), 17-23.
37. Okunji Chris O., Ayafor Johnson F., Jackson Joan E., Tally John D., Cyrus Bacchi, and Maurice M. Iwu, Brian G. Schuster, *In vitro* Antiprotozoal Activity of Quinolone Alkaloids from *Araliopsis Tabouensis* Aubrev & Pellegr. (Rutaceae) being abstract submitted for 7th International Congress of Ethnobiology (ISE) 23-27 October 2000 Athens, Georgia, U.S.A.
38. Okunji Chris O, Ware Tantalina A., Hicks Rickey P., Iwu, Maurice M., and Skanchy David J. (2001)., Capillary Electrophoresis Determination of Biflavanones from *Garcinia kola* in Three Traditional African Medicinal Formulations. *Planta Medica* (in press)
39. Onugu, A.J. 1998. "Capturing the Total Value of Natural Resources: Economic, Socio-cultural and policy perspectives, paper presented at the 12th Global Biodiversity Forum, Dakar, Senegal, December 1998.
40. Pearce, D.W., E. Barbier, and A. Markandya, 1990. "Environmental Sustainability and Cost Benefit Analysis". *Environment and Planning* 22: 1259-66.
50. Peters, C.M. 1994. "Sustainable Harvest of Non-timber Plant Resources in Tropical Moist Forests: An Ecological Primer". Biodiversity Support Programme, Washington, D.C. World Wildlife Fund.
51. Poulsen, G. 1982. "The Non-wood Products of African Forests". *Unasyuva* 34(137):15- 21.
52. Qu B. H., Thomas P. J., *J Biol Chem* 271, 7261 (1996).
53. UNEP/CONVENTION ON BIOLOGICAL DIVERSITY. 1996. "Economic Valuation of Biological Diversity". A Report of the Subsidiary Body on Scientific, Technical and Technological Advice. SBSTTA. Montreal, Canada: 2-6 September.
54. Rajaonarivony, J.I.M. 1996. "The Implementation of a Governmental Policy in Natural Products Research and Development in Madagascar". In: *Science in Africa: Utilizing Africa's Genetic Affluence through Natural Products Research and Development*. AAAS Symposium. Washington, D.C. AAAS Sub-Saharan Africa Program. Regan, T. 1981. "The Nature and Possibility of an Environmental Ethic". *Environmental Ethics* 3: 19-34.
55. Raymond M., P. Gros, M. Whiteway, D. Y. Thomas, *Science* 256, 232 (1992).
56. Riordan J. R., Rommens J. M., Kerem B. -S., Alon N., Rozmahel R., et al, *Science* 245, 1066 (1989).
57. Rommens J. M., Iannuzzi M. C., Kerem B-S., Drumm M. L., Melmer G., et al, *Science* 245, 1059 (1989).
58. Sheppard D. N., Rich D. P., Ostedgaard L. S., Gregory R. J., Smith A. E., et al, *Nature* 362, 160 (1993).
59. Sheppard D. N., Ostedgaard L. S., Winter M. C., Welsh M. J., *EMBO J* 14, 876 (1995).
60. Sunderland, T. and J. Nkefor. 1997. Conservation through Cultivation: A case study. The propagation of *Pygeum - Prunus africana*. TAA Newsletter. December 1997.
61. Sunderland, T., ML Ngo-Mpeck, Z. Tchoundjeu, and A. Akoa. 1999. The Ecology and Sustainability of *Pausinystalia johimbe*: an Over-Exploited Medicinal Plant of the Forests of Central Africa. CARPE/FAO.
62. Teem J. L., Carson M. R., Welsh M. J., *Receptors Channels* 4, 63 (1996).
63. Teem J. L., Berger H. A., Ostedgaard L. S., Rich D. P., Tsui L-C., et al, *Cell* 73, 335 (1993).
64. Thomas P. J., Shenbagamurthi P., Sondek J., Hullihen J. M., Pedersen P. L., *J Biol Chem* 267, 5727 (1992).
65. Tsui L. -C., *Trends Genet* 8, 392 (1992).
66. Welsh M. J., *FASEB J* 4, 2718 (1990).
67. WHO. 2001. New lease on life for resurrection drug. TDR News #64 (Feb. 2001) p. 18.